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A Review of the Application of Hollow-Fiber Liquid-Phase Microextraction in Bioanalytical Methods – A Systematic Approach with Focus on Forensic Toxicology

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ABSTRACT

In the past three decades, many studies employing hollow-fiber liquid-phase microextraction (HF-LPME) bioanalytical methods have been published. This type of extraction method presents many advantages over classical techniques, such as high preconcentration factor, clean extracts, and a green chemistry approach. The basic mechanism of extraction relies on the migration of the analytes through a liquid membrane sustained in the pores of the walls of a porous hollow fiber, and from there into an acceptor phase present in the lumen of the fiber. This acceptor phase can be injected directly onto the instrument used for analysis. The mass transfer occurs by passive diffusion and it can be enhanced by using a carrier or applying an electrical potential across the phases. Due to its advantages, and considering that no study systematically compiled the characteristics of the published methods in one single accessible source of information, the aim of this systematic review is to assess the data regarding bioanalytical methods, compile, and analyse the studies published until up to October of 2017. The data source used for the systematic review were Pubmed, Web of Science, and Science Direct, and 171 studies were included in the final review by two independent reviewers, resulting in a reliable and accessible source of information about bioanalytical methods employing HF-LPME.

KEYWORDS

Hollow-fiber liquid-phase microextraction, bioanalysis, forensic toxicology, sample preparation, green chemistry

1. INTRODUCTION

Sample preparation is a fundamental step in analytical chemistry, especially when the analytes are contained within complex matrices. The main purpose of this step is to simplify the matrix, enrich the analyte in the extract and clean-up the sample [1]. The extraction is an equilibrium-based process related to the distribution of a solute or solutes between two phases [2]. It is an essential step for many forensic toxicological analyses, which are normally related to complex biological matrices and low analyte concentrations [3, 4].

The most popular extraction methods are liquid-liquid extraction (LLE) and solid-phase extraction (SPE). However, these two conventional methods have limitations [3-5]. Even though LLE is a simple method with the capability of extracting many analytes at once, relatively large amounts of solvents are used, emulsions can be formed, and it is not ideal for volatile substances, since the extract must be evaporated [1]. SPE was introduced in the late 1970s and the pre-concentration of the analyte is normally poor and it can be highly time-consuming and laborious. Benefits of SPE however, include a relatively simple set up and clean extracts [3-5].

Over the years the scientific community has been trying to work on extraction methods requiring fewer steps within the process, and adaptable to field sampling and automation. The main purpose of this new arsenal of techniques and products is to provide faster, cheaper and cleaner methods for sample preparation. Moreover the scientists are focusing on methods with a reduced use of solvents due to the low environmental impact that an ideal solvent-free method would have. For that reason many of the methods are being miniaturized and presenting solvent-free approach. These methods are called microextraction methods [1, 6-8].

One of the microextraction methods is solid-phase microextraction (SPME), which consists of using a fiber coated with a sorbent to adsorb the analytes from the sample. This method is solvent-free, and gained acceptance and popularity amongst the scientific population, being used in many areas, such as forensics, environmental, and clinical. The technique is relatively fast and can be automated. Plus, many advances have been made related to SPME. On the other hand, this method also possesses drawbacks, such as the high-cost and limited lifetime of the fiber, and the carryover effect that may occur [3, 5, 9-11].

There are also the liquid-phase microextraction (LPME) methods that are basically a miniaturization of LLE: lower volumes of solvents are employed to extract the analytes from the sample, resulting in a reduction of solvent used and the analyst's exposure to potentially toxic substances. Many LPME methods were developed, including SDME, CFME, SBME, DLLME, and HF-LPME. These methods overcome many of the drawbacks of the aforementioned methods, being both fast and cheap [3, 5].

Many reviews have been written about HF-LPME [3, 11-24], but none of them have focused on forensic applications of the technique. The present review builds on theoretical and practical aspects of the technique, relating them to forensic toxicology. It includes a systematic review of the up-to-date bioanalytical methods published using this extraction process. It is also an up-to-date compilation of the bioanalytical methods using HF-LPME that can be used as a guide for future methods development in the field.

2. PRINCIPLES OF HF-LPME

The mechanism of the extraction using HF-LPME involves the partitioning of the analyte between two liquid phases. It depends on the partition coefficient and is influenced by many factors, such as: volume ratio between sample and extractor phase, pH of the phases, ionic concentration of the phases, extraction time, temperature, and forced convection of the system [3].

The device used to perform HF-LPME consists of a hollow porous fiber usually made of polypropylene (PP) (Figures 1 and 2) which permits the analytes from the sample to pass through into the lumen of the fiber, in which the acceptor phase resides. The fiber is immersed into the sample, which is aqueous and referred to as the donor phase. Before the extraction, the fiber is soaked in an organic solvent to fill its porous wall with the solvent. The fiber is then filled with the same organic solvent (2-phase HF-LPME) or with a different (and normally aqueous) acceptor solvent (3-phase HF-LPME). One of the ends of the fiber is connected to an apparatus, such as a syringe, to perform the step of introduction of the acceptor phase into the lumen of the fiber or its collection from it [13, 14, 16, 17, 25-27].

At a molecular level (Figure 2) the analytes are transferred by passive diffusion from the aqueous sample solution to the organic solvent in the walls of the fiber, and afterwards to the acceptor solution. The presence of the fiber wall provides some selectivity to the method, as it acts as a filter for high-weight molecules and particles, what provides an effective separation between the matrix and the analyte. The analytes can become trapped in the lumen of the fiber due to the ionization of the molecule (3-phase HF-LPME) or can be collected from the organic solution (2-phase HF-LPME). After the extraction, the extract is drawn inside a syringe and then injected directly into the analytical instrument, or it can be evaporated and reconstituted prior to injection [13, 14, 16, 17, 25-27].

Normally, the 3-phase HF-LPME is used for either basic or acidic analytes with ionisable functional groups, such as amines, phenols and carboxylic acids. The extraction, in this case, is based on acidic-basic equilibrium. Therefore, for the extraction of analytes with basic properties, the pH of the donor phase must be adjusted in an alkaline range, which will make the solubilization of the analyte in the aqueous solution more difficult, and will favour the transfer of the analytes to the organic phase within the walls of the hollow fiber. The pH of the acceptor phase, in this case, should be acidic to facilitate the delivery of the analyte to the acceptor phase, as the low pH would result in the analyte being in its ionized form, which is soluble in aqueous phases [1, 15, 16, 28-30]. If the injection into the analytical equipment is performed directly after the extraction, the 3-phase HF-LPME partially limits the analytical method to CE or LC [5].

Due to the disposable nature of the fibers the problem related to carryover present in SPME is eliminated. Also due to the capacity of the fiber to efficiently separate the matrix from the analyte, it is a good way of decreasing the matrix effects, and providing the cleaning-up of the samples, making it possible to use the extraction technique in complex matrices [20].

Moreover the ratio between the volumes of the donor and acceptor phases in HF-LPME is very high, making the enrichment factor of HF-LPME high [12, 31]. Besides that, this extraction method gives a high selectivity, is relatively simple, cheap, and is linked to green chemistry [7, 11, 12, 17]. Another advantage is that the technique combines extraction and concentration in one step, something that is not possible with other methods, such as LLE and SPE. On the other hand, it is usually not an exhaustive method and may present poor reproducibility due to manual cutting and sealing of the membrane [20].

3. PARAMETERS AFFECTING THE HF-LPME

During the development of a HF-LPME method some parameters can be optimized in order to obtain the best results. Some of these parameters are described below.

3.1 Solvent

To perform an efficient extraction, the solvent must present a good selectivity and a high partition coefficient related to the analyte to be extracted [6, 12, 15, 32]. The solvent can be chosen by the principle of like-dissolves-like. Most non-polar solvents (e.g. aliphatic or aromatic hydrocarbons) will efficiently extract most of the analytes via interaction through dispersion forces and hydrophobic effect. Solvents that present functional groups such as alcohol, ketone and halide may be used to increase the ability to interact through dipole-dipole or hydrogen-bonding interactions, increasing the extraction of more polar analytes. Therefore the choice of the solvent also depends on the characteristics of the analytes [1].

The viscosity is also an important parameter: the solvent must present an adequate viscosity to avoid leaking out of the fiber, however it cannot be too viscous to allow the diffusion of the analytes, improving the kinetics of the mass transfer [1, 12, 15, 32].

The loss of solvent due to evaporation and solubilization must also be taken into account. The loss of solvent can have a significant impact in HF-LPME due to the low volume of solvent used in the process (few microliters) [1, 12, 15, 32].

One important aspect of HF-LPME is the possibility of extracting and concentrating the analytes simultaneously, and therefore, choosing the optimal solvent is crucial. For example, 1-octanol is not directly compatible with the LC mobile phase, and needs to be evaporated and replaced by an adequate LC solvent prior to the injection if it is used as the acceptor phase. For GC, the extraction solvent must not co-elute with the analyte peaks, therefore they must present different boiling points [1].

The most common solvent used for LPME methods, including the HF-LPME, is 1-octanol. Many other solvents have been used, such as: toluene, tetrachloroethene, o-xylene, decane, 1-undecanol, dihexyl ether, n-hexane, undecane, and cyclohexane. Alkanes are generally not good as they cannot dissolve many of the analytes due to the absence of polar moieties [1].

3.2 pH of donor and acceptor phases

The effect of the pH has been discussed previously. Depending on the nature of the analyte (acidic or basic), the pH of the donor phase should be ideal to keep the molecules in the non-ionized form. In this form, the molecules are more soluble in organic solvents, increasing the migration of the analyte to the organic phase within the walls of the fiber. In case the system is a 3-phase, composed of another aqueous solution as the acceptor phase, the pH of that phase should be within a pH range that would keep the analytes in their ionized form, avoiding them being back-extracted to the organic solvent, and trapping them in the lumen of the fiber.

3.3 Ionic strength

Higher ionic strength (salt concentration) usually decreases the solubility of the molecules in aqueous solutions, and enhances the efficiency of the extraction. However, this effect is non-predictable and can be the opposite. The increase of the ionic strength of the aqueous solution also helps to decrease the solubility of organic solvents in it, diminishing the loss of solvent due to solubilization in the sample. On the other hand, the opposite effect of the addition of salt into the aqueous phase may happen due to the modification of the physical-chemical properties of the transition film present in the interface “donor phase-organic solvent” [1, 20].

This effect is called the salting-out effect and it varies with the type of salt employed (normally NaCl or Na_2SO_4) and, to a lesser extent, with the structure of the analyte. The difference in the propensity of the salt to cause salting-out is related to different characteristics of the ions (structure, size, charge density, hydration, and dielectric constant). It is important to highlight that the salting-out effect is an entirely physical phenomenon and does not affect properties of analytes or other molecules [1, 20, 33].

3.4 Extraction time

The process of extraction in systems with liquid-liquid equilibria involves the partioning between two liquid phases that are dynamically exchanging their contents between each other. Mass transfer is time-dependent and normally increases along with extraction. The HF-LPME methods normally do not run until it reaches the equilibrium as otherwise the extraction time would be too long, and part of the small amount of solvent would be lost during the process [20]. As equilibrium is normally not reached, extraction time is an important factor to be optimized, and the amount of analyte extracted will be dependent on the time expended in the extraction process.

3.5 Extraction temperature

Temperature is another factor that influences diffusion and constant of equilibrium of the species, resulting in a decrease in the time expended to reach the equilibrium. On the other hand, the water-organic solvent partitioning of most compounds is only weakly dependent on temperature, which means that temperature has little influence on the constant of equilibrium between liquid phases. However, it plays an important role when it comes to extraction involving water-gas or gas-solvent equilibria, e.g. headspace HF-LPME [1].

Even though higher temperatures can favour faster achievement of thermodynamic equilibrium, the increase of the temperature can cause evaporation of the solvent impregnated in the walls of the fiber, leading to loss of solvent [20].

3.6 Forced convection

Another important factor influencing distribution ratio is the mechanical energy input into the system. The molecules in solution undergo random motion, with constant changing speed and directions due to collisions and interactions with solvent molecules. The rate of transport of the molecules of analytes from one phase to another depends, at a molecular level, on this random molecular motion or diffusion. This diffusion is governed also by temperature of the system, viscosity of the environment, and size of the diffusing molecule. The convection process, normally done by stirring or agitation, is important as it enhances molecular diffusion, thus increasing the mass transfer between phases [1, 2].

Even though thermodynamic equilibrium time is inversely proportional to the mechanical energy submitted over the system, excess of agitation can cause the formation of bubbles on the surface of the fiber, decreasing the contact between the organic solvent and the analytes present in the donor phase. It also can cause evaporation of the organic solvent to be increased. Thus, optimum agitation intensity must be employed [34-37].

3.7 Use of a carrier or difference of voltage

HF-LPME is more efficient to extract analytes of low or moderate polarity. When it comes to the extraction of high polarity molecules, extraction efficiency cannot rely only on the partition coefficient of the analytes. In these cases, the experimentalist can use tools, such as the already cited acid-base speciation, and molecular pairing or application of an electrical field (EME). Another common solution is the adoption of pre-steps, such as derivatization [1].

The pairing molecule (aka carrier or surfactants) are amphiphilic molecules with a polar or ionic group and a hydrophobic group [38]. By converting the analyte in chemical species with higher partition coefficient than the original compound, the pairing-molecule improves the analyte extractability. The principle of carrier-mediated membrane transfer is to add an ion-pair reagent that generates a pair with the target analyte with opposite charge, enabling the ion-pairs to possess higher partition coefficients and consequently a higher transfer rate between phases (Figure 3) [3, 39, 40].

The application of voltage over HF-LPME was found to increase extraction efficiency, giving origin to EME. The extraction takes place in the same device as for the conventional HF-LPME, apart from the use of a power supply and electrodes to apply the voltage on the different compartments of the system (Figure 4). For using difference of voltage instead of pH gradient as the driving force, steady state can be reached faster, providing the extraction with good recoveries and selectivity [86, 88, 117, 147][85, 94].

4. REVIEW OF THE APPLICATION OF HF-LPME TO BIOANALYTICAL METHODS – A SYSTEMATIC APPROACH

4.1 Method

A review was completed to summarize the bioanalytical methods developed applying HF-LPME. For this purpose, the search covered three literature databases (Pubmed, Web of Science, and Science Direct) using the following search term: “microextraction AND hollow”. No search filters were used, except for Science Direct (in which we restricted the descriptors for the title, keywords and abstract only). The snowballing technique was also employed in the search of articles of interest. The aim was to carry out a wide literature search. All articles containing bioanalytical methods (analysis of any xenobiotic and biotics in biological systems) were included in the first step of the review. No exclusion criteria concerning the date of the publication were applied (the search was performed for relevant studies up to October of 2017). The exclusion criteria applied to reduce the initial pool of found articles to a final amount of articles that were included in the systematic review is summarized in Table 1 below.

Criteria related to the publication	Language of publication is not English
	Article was not available as full-text
Criteria related to the extraction method	Not HF-LPME
	Dynamic HF-LPME
Criteria related to the purpose of the study	Review article
	Pharmacokinetic study
	Protein-binding investigation
	Octanol/water distribution investigation
Criteria related to the sample	Analysis of environmental samples (e.g. water, soil)
	Analysis of food (e.g. vegetables, milk)
	Biological sample not from human source (e.g. rat blood)
Criteria related to the analyte	Analysis of metals and related compounds (e.g. organometallics)
	Analysis of compounds from the environmental exposure (e.g. substances from pollution of air or water)
	Analysis of compounds from dietary exposure (e.g. nitrites, preservatives)
	Endogenous substances with no forensic interest (e.g. angiotensin, vitamins, hormones, non-exposure biomarkers)
	Other substances with no forensic interest (e.g. cosmetics)

Table 1. Exclusion criteria applied to the review.

Studies with a different main purpose than developing methods using HF-LPME (such as protein-binding investigation) were included if they involved the development of a new HF-LPME method. Articles not available as full-text were attempted to be accessed by alternative sources before being excluded, such as inter-institutional request, author e-mailing, and search using other databases or Google.

Dynamic HF-LPME studies were excluded based on the different kinetics and dynamics of the system, as fresh solvent or sample is constantly introduced during the extraction in this technique.

Two reviewers independently completed the review, searching and compiling the articles. After this, they extracted the data from the articles in pre-defined tables. In case of difference between the data extracted by the reviewers, the discordant data were discussed, and agreement reached before data was adopted.

All of the data of interest was extracted, compiled (Tables 2 and 3) and discussed below.

4.2 Results

In the first step of the systematic review, 1002 potentially relevant articles were identified, of which 643 were excluded by simply reading the article title or abstract. From the remaining, 359 articles were evaluated and 188 articles were excluded upon reading the full text. The data of interest from the remaining 171 articles were compiled (Figure 5) in Tables 2 and 3 and discussed. Some articles presented more than one HF-LPME method.

4.3 Forensic Toxicological Applications of HF-LPME

Forensic toxicology plays an important role within the forensic sciences, and society has increasingly higher expectations of what forensic scientists in general can achieve. This is mainly due to the so-called "CSI-effect", but with the increasing number of cases related to NPS, forensic toxicologists are under considerable pressure to test for an increasing range of drugs in smaller and smaller specimen volumes [41-43]. Besides the NPS, several other drugs can be detected in biological samples related to legal proceedings, such as ethanol, cocaine, benzodiazepines and other sedatives, opiates and LSD and other hallucinogenic drugs. A great concern linked to all drugs potentially related to legal proceedings is the high heterogeneity between the toxicokinetic and physical-chemical properties of some of them, resulting in a challenge for the forensic toxicologists trying to analyse them, including the preparation of the samples. Forensic Toxicologists aim to develop sample preparation methods that are simple, cheap and efficient, and HF-LPME is an excellent extraction method option that has not been extensively explored in the field of forensic toxicology.

Extensive studies of the applicability of HF-LPME in the environmental field has been performed [20]. However the number of published studies involving the analysis of drugs in biological samples is considerably lower. For applications in forensic toxicology, the number of publications is further reduced. Tables 2 and 3 summarize, in chronological order, applications of two and three-phase HF-LPME for biological sample analysis, respectively.

4.4 Two-phase HF-LPME

Forty-one bioanalytical methods that fit into our inclusion criteria have been developed to analyse drugs using 2-phase HF-LPME (Table 2); the first study was developed in 2000 by Rasmussen et al. [44].

Biological matrices

Most of the studies used urine or plasma as matrices (30 and 21 out of 41 respectively) [5, 44-80]. In post-mortem forensic toxicology the most commonly used biological specimen is whole blood, however from all of the 2-phase HF-LPME studies, only 2 used whole blood [71, 81]; limited detail was provided regarding the sample preparation in these studies. Two articles used oral fluid [75, 82]; one of them [82] developed the method to extract cocaine and its metabolites, analytes commonly identified in forensic toxicology casework. The detection of drugs of abuse in oral fluid is becoming more common in workplace drug testing, and the method developed in this study resulted in a fast and sensitive method (10 minutes long and limits of detection of 6-28ng/mL by GC-PD-HID). Similar extraction conditions were applied to screen urine for the same analytes in another study [46], however the extraction time was longer when oral fluid was used, probably due to the lower amount of sample available when compared to urine, and the lower concentration of the drugs in oral fluid. On the other hand, the method provided higher reproducibility for oral fluid compared with urine. Three studies described hair methods utilizing 2-phase HF-LPME [60, 73, 83] but the sample preparation was more complex. Some studies mentioned the difference in viscosity and protein-binding as reasons for different recoveries when using different biological matrices [45, 53]. Most of the studies used the pure biological material or just a normal dilution or filtration, with no previous step, however one study [63] used protein precipitation in the sample preparation prior to HF-LPME, and demonstrated that this approach can be a useful tool for cleaning up the sample and releasing the analytes.

Ionic strength

Thirty-two out of the 41 publications included studies mentioned to have used ionic strength as one of the optimized parameters [5, 45, 49, 51-55, 57-60, 62-70, 72-81, 83]. Eighteen of them decided not to use the salting-out technique in the final method [45, 49, 51, 53, 55, 57, 58, 63, 64, 69, 70, 72-74, 77, 78, 80, 81] due to either the negative impact of increased salt concentration on the extraction or the lack of effect of it; and 14 of them [5, 52, 54, 59, 60, 62, 65-68, 75, 76, 79, 83] decided to use the salting-out effect to improve extraction. All the studies that employed the salting-out effect, with exception of three [59, 75, 76], used sodium chloride. Meng et al. [75] saturated the donor phase with different salts, and Na_2SO_4 presented best results even though the concentrations that were compared were different. The concentrations of salt varied from 1% (w/v) to approximately 30% (w/v). Nine of the 41 studies [44, 46-48, 50, 56, 61, 71, 82] did not mention ionic strength as a parameter to be optimized.

Ion pairing

Ion pairing was used in only four out of the forty-one 2-phase HF-LPME [47, 70, 78, 80] studies. Kramer et al. [47] compared two approaches: to extract the analyte (THC metabolite) by acidifying the sample, making the analyte neutral, and not using an ion-pairing agent; or to basify the sample and use an ion-pair agent to form neutral species with the ionized analyte. The second approach presented far better results in terms of peak area, and therefore was adopted to perform further extractions. According to the authors the higher ionic strength of the donor phase due to the ionization of the analyte and due to the presence of the ion pairing salt also contributed to less leakage of solvent from the fiber.

Fiber

Most of the 2-phase HF-LPME systems were built using PP fibers with an internal diameter of 0.60mm, wall thickness of 200μm, pores of 0.2μm, and 70% porosity. However, some studies used PVDF [53, 65, 69, 72] or polyethersulfone fibers [75], or PP fibers with different dimensions [47, 54]. In 2001 [46] a group of researchers employed different fibers to extract cocaine and metabolites from urine, and PP presented better performance (larger peak areas and more reproducible results) than PVDF. No further information was given about the reason for the difference between the results using different fibers. Cui et al. [53] compared PP and PVDF fibers, both differing only in wall thickness and porosity. The PVDF fiber presented a better solvent compatibility and faster extraction efficiency and operational accuracy due to its higher porosity. The thickness of the wall is an important factor that influences extraction efficiency and equilibrium time. Another study compared different fibers [65], two PVDF and one PP (all with different dimensions), and also attributed the better results of the PVDF fiber to its higher porosity. The porosity of the PP fiber was not cited and the wall thickness, although is an important parameter, was not explored in this study. An overall conclusion is that the dimensions of the fiber impacts on the extraction efficiency; solvent permeability and extraction time are highly influenced by the porosity and pore size. The wall thickness plays an important role in the equilibrium time (the thicker the wall is, the longer it takes for an equilibrium to be reached, as the wall decreases the speed of mass transfer between the sample matrices and extraction solvent) [84]. Leinonen et al. [51] noticed a wide difference among the extraction efficiency of anabolic steroids (some analytes were not extracted at all) between LLE and HF-LPME, and the reason for the difference is the high adsorption of the analytes to the PP, therefore the material of which the fiber is made is another factor that should be considered in the choice. One of the studies used a polyethersulfone home-made fiber for HF-LPME [75], and the recovery results presented were very good (recoveries>90%).

Solvent

Different organic solvents were used to impregnate the fiber and as acceptor phase. Most of the solvents are long-chain alcohols (1-heptanol, 1-octanol, 1-nonanol, and 1-undecanol). Some solvents with high extractability performance are too volatile to be used in a microextraction method. An option to overdraw this issue is to use less volatile solvents as co-solvents (approach adopted by Sun et al. [69]). Other studies also used a mixture of solvents to extract the analytes [45, 53, 72]. In 2013 [64], a research group used the so-called supramolecular solvents (see reference for details) for HF-LPME extraction for the first time, and it proved to be suitable to substitute organic solvents in 2-phase HF-LPME procedures which also reinforces the green chemistry aspect of HF-LPME [17].

Derivatization

All studies that used derivatization [47, 54, 74, 75] concluded that in-tube/in-situ derivatization - happens within the walls of the fiber during the extraction - can be used instead of the traditional derivatization procedure - takes place in a separate vial. Kramer et al. [47] compared both derivatization methods and obtained poorer extraction efficiencies with in-tube derivatization; the reason is the mild temperature and short time employed by the authors, who justified the lower performance stating that the ease and speed of the method compensates for this decrease. Liu et al. [54], on the other hand, obtained better results for in-situ derivatization, and one of the reasons could be the longer extraction time and higher temperature employed in this study.

Forced convection

All 2-phase HF-LPME were performed under forced convection (stirring, shaking/vortexing/vibrating, or ultrasonicating). Around 80% of the articles used stirring with a magnetic stir bar to force convection onto the system. Liu et al. [67] introduced ultrasonication in the extraction process aiming to enhance the extraction throughput and fasten the extraction time by improving mass transfer. They compared the extraction time to achieve the equilibrium using stirring or ultrasonication under the same conditions (salt concentration, pH, and temperature), and the process using ultrasonication achieved equilibrium after 10 minutes, whilst the system using stirring reached equilibrium after 30 minutes. However, the use of ultrasound irradiation must be carefully evaluated due to the possibility of damaging the fiber [53].

Extraction time

Most of the extraction processes were between 10 and 30 minutes (17 studies) or 30 to 60 minutes (16 studies). One study [82] aimed to develop a rapid screening test for cocaine and metabolites in urine, therefore employing a short extraction time (3 minutes). On the other hand, some studies had a long extraction time (equal or higher than 60 minutes) aiming to achieve exhaustion and avoid having a kinetic method [50, 65, 68, 70, 80].

Temperature

Few studies adopted extraction temperature as an optimizable parameter. Most of them performed the extraction at room temperature. Few studies adopted temperature higher than 30^{o}C after optimization [52, 54-56, 59, 60, 62, 67, 74, 76]. Most of these studies justified the positive effect of temperature on the extraction based on kinetics and thermodynamics of mass transfer; only three studies [54], [74] and [56] justified the higher extraction efficiencies under higher temperature based on the improvement of derivatization and hydrolysis efficiencies respectively.

Electromembrane extraction

Only two 2-phase HF-LPME studies [61, 73] used EME. EME was first used in 2006, and it is widely employed in 3-phase systems in which both the donor and acceptor phases are normally aqueous. The solvents must present some important characteristics in EME, such as good electrical permittivity and potential to dissolve ionic species. Daravani *et al*. [61] compared the EME of a model analyte in 2- and 3-phase HF-LPME, and concluded that the 2-phase HF-LPME proved faster and simpler. By using a combination of EME and 2-phase HF-LPME, they concluded that mainly electrokinetic migration was responsible for the extraction, and not simple passive diffusion, as the equilibrium was achieved in a short period of time.

Analytical method

The analytical instrumentation systems employed for separation and detection or quantification of the analytes were very variable (GC, LC, CE and ULC with NPD, PD-HID, FID, UV, MS, MS/MS, FPD, ECD or FD). For the 2-phase HF-LPME, GC was the most used technique for separation due to the nature of the acceptor phase being organic. Some of the studies that used LC or ULC [49, 51, 74] for separation had to dry the extracts and reconstitute them with the appropriate solvent before injecting onto the instrument. Other used a small injection volume (5µL or 10µL) not to disturb the chemical equilibrium between the mobile phase and the analyte [52, 69, 76]. Five studies [59, 68, 70, 77, 80] did not adopt any of these approaches but still obtained good results from the LC-FD and LC-UV. The study that used supramolecular solvent also injected a relatively high volume (20µL) of extract onto the LC-UV [64]. One study [65] took 10µL of the acceptor phase (1-octanol), and diluted with 300µL of methanol before the injection onto the LC.

Ref.	Analytes	Matrix	Donor phase: Volume pH Additives	Fiber: Material Length id(mm)xwt(mm)xps(μm)	Solvent and Additives	Acceptor phase and Additives	Extraction process	Instrumentation
[44]	Diazepam Prazepam	U P	1.5mL pH 5.5	PP 4cm 0.6x0.2x0.2	1-octanol	1-octanol	Vibrating 1000rpm 30min	GC-NPD
[45]	Diazepam NDMD	U P	3.5 (U); 3.0mL (P) pH 7.5 No salt added	PP 6cm 0.6x0.2x0.2	Butyl acetate:1-octanol (1:1 v/v) (U) Dihexyl ether:1-octanol (1:3 v/v) (P)	Butyl acetate:1-octanol (1:1 v/v) (U) Hexyl ether:1-octanol (1:3 v/v) (P)	Vibrating 600rpm 50min	GC-NPD
[46]	Cocaine Cocaethylene EMeE AEME	U	8mL pH 10.6	PP 6cm 0.6x0.2x0.2	Chloroform	Chloroform	Stirring 1600rpm 3min	GC-PDHID
[47]	THC-COOH	U	8mL pH 8 Bu_4N^+-HSO_4	PP 6cm 0.6x0.2x0.64	Octane:BSTFA (1:5 v/v)	Octane:BSTFA (1:5 v/v)	Stirring 1540rpm Room T 8min	GC-PDHID
[82]	Cocaine Cocaethylene EMeE AEME	OF	2.2mL pH 10.5	PP 7cm 0.6x0.2x0.2	Chlorophorm	Chlorophorm	Stirring 2000rpm 10min	GC-PDHID
[48]	Methadone Promethazine Haloperidol	U P	4mL pH 13.1	PP 8cm 0.6x0.2x0.2	Dihexyl ether	Dihexyl ether	Vibrating 1500rpm 45min	GC-FID
[49]	Mirtazapine	P	4mL pH 13.6 No salt added	PP 7cm 0.6x0.2x0.2	Toluene	Toluene	Stirring 30min *ca.* 22^{o}C	LC-UV
[50]	Basic drugs	P	pH 7	PP 6.5cm 0.6x0.2x0.2	1-octanol	1-octanol	Vibrating 1500rpm 60min	CE-UV
[51]	Anabolic steroids	U	4mL pH 7 No salt added	PP 6cm 0.6xn.r.x0.2	1-octanol	1-octanol	Stirring 1250rpm Room T 45min	LC-MS
[52]	Thiazide diuretics Clopamide Probenecid Loop diuretics	U	7.5mL pH 2 15% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 1010rpm 40^{o}C 40min	LC-MS/MS
[53]	Flunitrazepam	P U	4mL pH 9.5 (U); 8.0 (P) No salt added	PVDF 1.8cm 1.2x0.2x0.2	p-xylene (U) p-xylene:1-octanol (3:7 v/v) (P)	p-xylene (U) p-xylene/1-octanol (3:7 v/v) (P)	Stirring 375 (P); 450 (U) rpm 30^{o}C 30min	GC-MS/MS
[54]	Clenbuterol Metoprolol Propranolol	U	5mL pH 12 14% (w/v) NaCl	PP 1.6cm 0.6x0.2x0.2	Methylbenzol	Methylbenzol:MSTFA (1:1 v/v)	Stirring 925rpm 35^{o}C 20min	GC-MS
[75]	Free cyanide	U OF	5mL pH 6.5 Saturated with Na_2SO_4	Polyethersulfone 1.5cm 3.75x0.75x0.2	Sodium carbonate + Ni(II)-NH_3	Sodium carbonate + Ni(II)-NH_3 pH 11	Stirring 900rpm Room T 10min	CE-UV
[83]	THC CBD	H	10mg pH 14	PP 6cm	Butyl acetate	Butyl acetate	Stirring 600rpm	GC-MS/MS

	CBN		6.8% (w/v) NaCl	0.6x0.2x0.2			Room T 20min	
[55]	Promethazine Promazine Chlorpromazine Trifluoperazine	U	3mL pH 9 No salt added	PP 1.5cm 0.6x0.2x0.2	Toluene	Toluene	Stirring 1000rpm 40°C 10min	GC-FPD GC-FID
[5]	Amphetamines Caffeine Ketamine	U	3mL pH 12.5 30% (w/v) NaCl	PP 1cm 0.6x0.2x0.2	o-xylene	o-xylene	Stirring 1000rpm Room T (30°C) 20min	GC-FID
[56]	Pyrethroid metabolites	U	5mL pH *ca.* 1	PP 1.5, 2.0, 2.5 and 3.0cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 70°C 10min	GC-ECD
[57]	Alfentanil Fentanyl Sufentanil	P U	3mL pH *ca.* 11 No salt added	PP 1cm 0.6x0.2x0.2	Dihexyl acetate	Dihexyl acetate	Stirring 1000rpm Room T (25°C) 15min	GC-NPD
[81]	Amitriptyline Imipramine Promethazine	B	30µL pH 11 No salt added	PP 1cm 0.6x0.2x0.2	Toluene	Toluene	Stirring Room T 10min	GC-MS
[58]	Tramadol	P U	12mL pH 12 No salt added	PP 1.5cm n.r.	1-nonanol	1-nonanol	Stirring 1000rpm Room T 25min	GC-MS
[59]	Guaifenesin	P	25mL pH 7.4 1.7% (w/v) K_2HPO_4	PP 8cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 600rpm 37°C 30min	LC-FD
[60]	Anabolic steroids	U H	20mL No pH adjustment 7.5% (w/v) NaCl	PP 1.2cm 1.8x0.2x0.2	Toluene	Toluene	Stirring 750rpm 40°C 30min	GC-MS
[61]	Imipramine Desipramine Citalopram Sertraline	U	1.2mL Neutral (pH *ca.* 7)	PP 2.2cm 0.6x0.2x0.2	1-heptanol	1-heptanol	Stirring 1400rpm 60V 15min	GC-MS
[62]	Sulfetanil Alfentanil	P U	5mL pH 10 15% (w/v) NaCl	PP 1.3cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 700rpm 50°C 25min	GC-FID
[63]	Fluoxetine Norfluoxetine	P	5mL pH 11 No salt added	PP 3.7cm 0.6x0.2x0.2	Dihexyl ether	Dihexyl ether	Vibrating 700rpm 30min	GC-MS
[64]	Benzodiazepines	P U	*ca.* 5 (P); *ca.* 25 (U) mL pH *ca.* 9 No salt added	PP 10cm 0.6x0.2x0.2	Supramolecular solvent	Supramolecular solvent	Stirring 900rpm 50min	LC-UV
[65]	Indomethacin Dexamethasone Propafenone	P U	1.8ml pH 2, 2-8, 10 20% (w/v) NaCl	PVDF 3.5cm n.r.xn.r.xn.r.	1-octanol	1-octanol	Vibrating 173rpm Room T 102, 120 and 102min	LC-UV
[66]	Methadone	P U	10mL pH 11.5	PP 2cm	1-undecanol	1-undecanol	Stirring 700rpm	GC-FID

			5% (w/v) NaCl	0.6x0.2x0.2			20°C 45min	
[67]	Nicotine	P	4.5mL pH 7.4 29% (w/v) NaCl	n.r. 3cm n.r.	1-octanol	1-octanol	Sonicating 37°C 10min	GC-FID
[68]	Amlodipine	U	24mL pH 10 1.2% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 1000rpm 60min	LC-UV
[69]	Naloxone Buprenorphine Norbuprenorphine	P	5mL pH 8.7 No salt added	PVDF 4cm 0.8x0.175x0.16	1-octanol:chlorophorm:toluene (2:4:4 v/v/v)	1-octanol:chlorophorm:toluene (2:4:4 v/v/v)	Stirring 1000rpm 20°C 30min	ULC-MS
[70]	Hydrochlorothiazide	U	24mL pH 12 No salt added	PP 8.5cm 0.6x0.2x0.2	1-octanol + 2% (w/v) Aliquat 336	1-octanol + 2% (w/v) Aliquat 336	Stirring 800rpm 90min	LC-UV
[71]	Amphetamines Methcatinone Ketamine Meperidine Methadone	U B	8mL pH 13	PP 4cm 0.6x0.2x0.2	Toluene	Toluene	Stirring 500rpm 30°C 15min	GC-MS
[72]	Flunitrazepam	P U	4mL pH 9.5 (U); 8.0 (P) No salt added	PVDF 1.8cm 1.2x0.2x0.2	p-xylene (U) p-xylene:1-octanol (3:7 v/v) (P)	p-xylene (U) p-xylene:1-octanol (3:7 v/v) (P)	Stirring 375 (P); 450 (U) rpm 30°C 30min	GC-MS
[73]	Metamphetamine	H U	4mL pH 7 No salt added	PP 6cm 0.6x0.2x0.2	1-octanol + 2.5mg/mL grapheme oxide	1-octanol	Stirring 1000rpm 60V 20min	GC-FID
[74]	Memantine	P	10mL pH 13 No salt added	PP 8cm 0.6x0.2x0.2	Cyclohexane	Cyclohexane + 0.3mg/mL dansyl chloride + 4% (v/v) triethylamine + 10% (v/v) acetone	Stirring 800rpm 40°C 50min	LC-FD
[76]	Naproxen Nabumetone	P U	pH 3 KCl 4% (w/v)	PP 4cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Stirring 600rpm 45°C 20min	LC-FD
[77]	Albendazole Triclabendazole	U	pH 8 No salt added	PP 8.8cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Vortexing Room T 3min	LC-FD
[79]	Oxazepam Lorazepam	U P	25mL No pH adjustment 7.5% (w/v) NaCl	PP 10cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 1000rpm 50min	LC-MS
[80]	HCTZ	U	24mL pH 12 No salt added	PP 8.5cm 0.6x0.2x0.2	1-octanol + Aliquat 336 2% (w/w)	1-octanol + Aliquat 336 2% (w/w)	Stirring 800rpm 90min	LC-UV
[78]	Warfarin	P	8mL pH 6.5 No salt added	PP 3cm 0.6x0.2x0.2	1-octanol + CTAB 10mM	1-octanol + CTAB 10mM	Stirring 800rpm Room T 25min	UV-Vis

Table 2. 2-phase HF-LPME (conventional and variants) of drugs of forensic interest in biological matrices. The concentration values of salt added were converted to % (w/v); the pH were calculated based on the concentration of base or acid in some cases. Abbreviations: (A) = acidic; AEME = anhydroecgonine methyl

ester; Aliquat-336 = 3-caprylil methyl ammonium chloride; (B) = basic; B = whole blood; BSTFA = bis(trimethylsilyl)trifluoroacetamide; Bu_4N^+-HSO_4= tetra-n-butylammonium; *ca*. = approximately; CBD = cannabidiol; CBN = cannabinol; CE = capillary electrophoresis; ECD = electron capture detector; EMeE = ecgonine methyl ester; FD = fluorescent detector; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; H = hair; id = internal diameter; K_2HPO_4 = dipotassium phosphate; LC = high performance liquid chromatography; MS = mass spectrometry; MS/MS = tandem mass spectrometry; n.r. = not reported; Na_2SO_4 = sodium sulfate; NaCl = sodium chloride; NDMD = N-desmethyldiazepam; NPD = nitrogen-phosphorus detector; OF = oral fluid; P = plasma; PDHID = pulsed-discharge helium ionization detector-helium ionization detector; PP = polypropylene; ps = pore size; PVDF = polyvinylidene difluoride; T = temperature; THC-COOH = 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid; THC = Δ9-tetrahydrocannabinol; wt = wall thickness; U = urine; ULC = ultra-high performance liquid chromatography; UV = ultra-violet

4.5 Three-phase HF-LPME

One hundred and forty-two bioanalytical methods were described in 136 publications fit into the inclusion criteria (Table 3).

Biological matrices

Similarly to 2-phase HF-LPME, most of the 3-phase HF-LPME methods included in the systematic review used plasma (92 out of 142) and/or urine (99 out of 142) as the matrix. Few methods used other matrices, such as whole blood [85-92], breast milk [93-96], serum [97, 98], oral fluid [99, 100], hair [101], or liver [102].

Halvorsen et al. [85] demonstrated that despite the complexity of whole blood as a matrix the recoveries obtained for whole blood and plasma were similar during the extraction of methamphetamine and citalopram. However, the time to reach the equilibrium was twice as long for whole blood. The same group [86] of researchers tested a screening test for amphetamines, and the LOD observed for urine were higher than the LOD observed for whole blood due to the high noise present in urine samples. They [87] also evaluated qualitatively the matrix effects using the HF-LPME combined with LC-MS for plasma and whole blood, and no ion suppression was observed due to the effective sample clean-up in HF-LPME. Indeed the HF-LPME provided a good clean-up for the whole blood without any complex pre-treatment of the sample in all the studies. Gjelstad et al. [89] showed that the dilution of whole blood per se tends to improve the efficiency of the process, making the recovery of basic drugs similar to their recovery from plasma. All of the studies used simple dilution to deal with whole blood samples.

Low recoveries (18-38%) previous to any pre-treatment were obtained for breast milk [93] in one study, and the authors believe this is due to the rate of drug binding to the sample matrix. The authors tested different pre-treatments, and after centrifugation or acidification followed by centrifugation the recoveries improved, reaching rates up to 69%. By using EME, Kjelsen et al. [94] obtained good recoveries from breast milk (comparable to the recoveries obtained from plasma and urine). The recoveries of levimasole using oral fluid as the biological matrix were similar to those obtained from other matrices [100] using EME. Comparable recoveries were also obtained for lidocaine and chlorpromazine from serum and urine in two studies [97, 98].

One study used hair to analyse amphetamine-type stimulants [101] while another study [102] used liver samples to analyse barbiturates, and due to the complexity of dealing with these matrices, the sample preparation is more complex and detailed in the articles.

Ionic strength

From the 136 studies, 51 did not optimize ionic strength. Sixty out of the 142 methods [35, 37, 38, 77, 97, 99, 100, 103-154] decided not to use salt after optimization (some studies have more than one method, therefore the number of references can differ from the number of methods), and 27 used NaCl with concentrations ranging between 1% to around 30% (w/v). One study used KCl for salting-out effect, and 3 studies [75, 155, 156] used Na_2SO_4 at different concentrations. Two studies compared Na_2SO_4 with different salts; Lv and cols. [155] compared NaCl and Na_2SO_4 at the same concentrations, and the sulphate presented better results, however, because the ionic strength is related to the concentration of all the ions in solution, Na_2SO_4 is ionically stronger when in solution, and comparing both salts in the same concentration is not the same as comparing their ionic strength. Moreover this study used a carrier and the decreased interaction between the elements of the biological sample and the carrier by the use of a salt could be the reason for the increase in extraction efficiency.

Ion pairing

The use of ion-pairing agents was present in only 28 methods (27 studies) of the 142 methods [38-40, 50, 79, 99, 100, 103, 105, 119, 120, 126, 131, 140, 146, 152, 153, 155, 157-165]. Aiming to correlate solubility and log D data with extraction recoveries, Pedersen-Bjergaard et al. [50] tried different HF-LPME methods (2-phase HF-LPME and 3-phase HF-

LPME with and without carrier) to extract drugs with different chemical behaviour. They found that those drugs with water solubility values above 150mg/mL and log D<1.8 at pH 13, carrier-mediated HF-LPME should be the preferred technique. The same research group performed other studies using carrier agents. One of these studies [40] compared different organic borates, phosphates, sulphates, and carboxylic acids as pairing agents to extract hydrophilic basic drugs; the carriers tested were both water soluble and not, therefore, some were solubilized in the donor phase and some of them in the solvent. An important conclusion of this study is the demonstration of non-compatibility of some carriers with biological samples due to the precipitation of proteins on the surface of the fibers and in the donor phase. They also showed that by saturating the biological sample with sodium sulphate it is possible to increase the recoveries using a carrier due to the suppression of the interactions between the carrier and plasma proteins that this agent provides. Aliquat-336 was used in approximately one fifth of the 3-phase HF-LPME studies that used a carrier agent (6 out of 28), and 10 studies used TEHP, DEHP or both. Combination with EME happened in some studies [99, 100, 105, 126, 140, 146, 159, 162, 165]. A recent study [105] used C60 fullerene as a carrier agent for the first time.

Fiber

Most of the articles that described the fibers used in the extraction procedure used PP fibers with the conventional parameters already cited; some articles that used PP fibers with other dimensions [50, 85, 87-89, 93-96, 130, 151, 154, 166-172] or fibers made of different material, such as PVDF [129]. Xi et al. [169] compared fibers made of different materials and with different dimensions (PP, PVDF, polysulfone, and polyethersulfone). The PP fiber with 0.45mm wall thickness and 0.18μm pore size presented the best results. Without any further information about porosity percentage and wall thickness of the PVDF, polysulfone and polyethersulfone fibers it is not possible to properly correlate the best performance of the PP fiber to its dimensions and composition. Halvorsen et al. [85] showed that by changing the dimensional parameters of the hollow fibers it is possible to obtain a considerably shorter equilibrium time, mainly when complex biological matrices are used. This happens when the contact area is increased and the wall thickness is reduced. As expected, the differences between the recoveries were not significant after the equilibrium was reached.

Solvent

The most common solvent used to impregnate the hollow fiber was 1-octanol (49 studies), followed by dihexyl ether (28), NPOE (20), and aliphatic hydrocarbons (16). Other solvents consisted of other long-chain aliphatic alcohols, esthers, silicon oil, etc. Some studies aimed to develop green bioanalytical methods by testing essential and fixed oils from plants. Four studies [40, 102, 167, 173] used plant oils in their extraction method and obtained good results, and this reinforces the idea of the green chemistry already at the core of the concept of HF-LPME. One of the studies [167] tested different fixed (almond, arachis, olive, and soy-bean oils) and essential oils (anise, fennel, lavender, and peppermint oils) and compared with traditional HF-LPME solvents (dihexyl ether, 1-octanol, and dodecyl acetate) to extract the same group of analytes; the results were in general similar between the essential oils and the traditional HF-LPME solvents. The fatty oils presented worse results probably due to their high viscosity, which lowers the diffusion rate across the organic phase. It was not possible to immobilize eucalyptus, lemon, tea tree, clove, and thyme oil, and also oil of turpentine in the walls of the fiber. Ho et al. [40] compared the carrier-mediated extraction of 8 drugs from plasma using peppermint oil or conventional solvents (1-octanol, dihexyl ether, NPOE, 2-octanone, dodecyl acetate and silicon oil AR20); seven out of 8 drugs had higher extractions using peppermint oil. Menck et al. [102] also tested different fixed and essential oils to extract barbiturates from liver samples, and even though eucalyptus oil did not present the best results for all the analytes, it was adopted as the best option due to its good performance. Eucalyptus oil was also chosen as the best option for extracting ketamine and its metabolites from urine in other study [173].

Derivatization

Four studies used in-situ derivatization during the 3-phase HF-LPME [51, 115, 142, 149]. Derivatization played different roles in the included studies, it was used to make a compound susceptible to analysis using GC by increasing its volatility [51], to create a chromophore making the compound possible to be analysed by UV [115], or just to make the compound less hydrophilic to facilitate its migration to the solvent [142, 149]. Also the derivatization took place in different places in the system: in the fiber [51], in the DP during the extraction [142, 149], or in the DP previous to the extraction [115]. The adoption of relatively harsher conditions (45^{o}C for 30 minutes) employed by Leinonen et al. during the extraction is based on the need of this condition for derivatization. Relatively low recoveries were obtained in this study due to the sensitivity of sylilation to the water present in the sample; the authors tried to overdraw this by the use of dihexyl ether as the solvent layer membrane to protect the reaction that happened in the lumen of the fiber.

Forced convection

Most of the methods used stirring (104) or vibration/vortexing/shaking (25) to force the convection of the system. Three studies used sonication for this purpose [90, 102, 174]. Four studies did not provide the system with any agitation method [91, 168, 169, 175]. Eibak et al. and Jamt et al. tried to simplify the extraction method by not stirring the system; Xi et al. did not use any convection method to avoid influencing the drug-protein binding. One study used magnetofluid to stir the system [176], and that provided the method with shorter extraction time (8 minutes). No study directly compared different agitation methods, and this is a potential field to be explored by future researchers.

Extraction time

Regarding the extraction duration, some studies presented short extraction time (equal or less than 5 minutes) [77, 91, 94, 130, 168]. Apart from one study [77], all of them used EME, what explains why the methods were shorter. Even though two other studies [90, 102] presented relatively short extraction time (5 minutes), time was not an optimized parameter; these studies used ultrasonication as a forced convection method. Eibak et al. [168] presented for the first time an EME kinetic method, and it was used to effectively quantify within 1 minute amitriptyline, citalopram, fluoxetine, and fluvoxamine in human plasma. Song at al. [176] developed and validated a method to quantify aristolochic acids in human plasma and according to the authors the short extraction time (8 minutes) was due to the use of magnetofluid during the magnetic stirring of the samples. Eskandari et al. [122] showed a significant extraction time decrease (from 60 to 15 min) by adopting EME instead of the conventional HF-LPME. Other studies also compared EME to HF-LPME [122, 159, 175].

On the other hand some studies presented a long extraction time (equal or greater than 60 minutes, reaching up to 5 hours) after optimizing this parameter [68, 70, 80, 88, 103, 110, 118, 120, 122, 136, 151, 161, 169, 171, 172, 177-179]; other studies adopted long extraction times but did not optimize it [40, 50, 93, 157]. Halvorsen et al. [85] showed that by increasing the contact surface between the solvent impregnated within the fiber walls and the donor and acceptor phases it is possible to decrease the extraction time (in this case by a factor of 2). Xi et al. [169] adopted a 5-hours extraction method due to the stagnant characteristic of the system (the aim of the study was to determine the protein-binding properties of the drugs). According to the central composite design by Ebrahimzadeh et al. [180] the extraction time did not play an important role in his method, however, they adopted a long extraction time to ensure equilibrium was reached. One study presented a total extraction time of 60 min [149] but it was a sequential extraction of two drugs with different systems in the same vial. Some studies justified the long extraction time based on the complex nature of the biological matrices [85, 88].

Temperature

Temperature was optimized in 12 methods [34, 51, 92, 106, 117, 124, 129, 142, 178, 181-183]. Two of them [51, 142] explained the use of higher temperatures to perform rapid and higher derivatization. The general behaviour of the extraction was the increase of the extraction efficiency, and after achieving an optimum temperature, the decrease of the efficiency. The main reasons for this could be solvent depletion (due to easier solubilization of the solvent in the donor phase at higher temperatures, or due to volatilization), and fiber damaging. An important factor to be considered is the Joule effect that can happen during the EME, which can increase the temperature of the system.

Electromembrane extraction

Thirty-one methods used EME to perform their extractions [89, 91, 94-96, 99, 100, 105, 122, 126, 127, 130, 132, 140, 141, 144, 146, 151, 152, 154, 159, 160, 162, 165, 168, 175, 184-188]. From those, 20 used NPOE as the extraction solvent (with or without a carrier), and according to these authors this solvent is already well established as a good option for EME for extracting basic compounds; 1-octanol, a traditional solvent for HF-LPME is less likely to be used with EME due to the formation of bubbles under high voltage [130]. One of the advantages of using EME is that it usually does not require sample pre-treatment [89, 91, 140, 168, 175]. Some studies showed that the kinetics of the EME can be slower when applied to biological matrices, potentially leading to a lower recovery after the same extraction time [89, 94, 159, 175, 184]; this is probably due to protein binding and higher viscosity of the biological samples. The performance of EME was compared to the performance of conventional HF-LPME in some studies [122, 151, 159, 175, 184], and EME proved to be faster and more efficient in general. EME showed to be effective even for stagnant systems and short extraction times [168, 175]. Daravani et al. [130] were the first to try to extract acidic compounds from complex biological matrices using EME. After this study other studies included acidic compounds, one of them [140] using 2 fibers in the same system to extract basic and acidic substances simultaneously. For that Seidi et al. impregnated the different fibers with different solvents that presented optimum extraction for both types of drugs. Other studies compared sequential and simultaneous extraction of acidic and basic substances, and the sequential option presented better performance [96].

Koruni et al. [146] also used different fibers and different systems to extract acidic and basic drugs with a wide range of log P, and for that four systems were used for simultaneous extraction. Koruni et al. [146] adopted and interesting approach to analyse basic and acidic compounds simultaneously by using a set of two auxiliary electrodes and hollow fibers. Eibak et al. [175] used multiple fibers, however, the aim was not to simultaneously extract drugs with different properties but to demonstrate how an increase in the SLM contact area and acceptor phase volume could impact the extraction efficiency. The objective of the study was to achieve exhaustive extraction (recoveries higher than 95%) in a short period of time by this geometry optimization of the HF-LPME system, and also to test different extraction procedures (by changing volume of donor phase and convection process). Even though they succeeded in achieving an exhaustive extraction from water, the same was not observed when extracting from plasma, probably due to the protein binding. Moreover, even using the 3-fibers system, only one out of six drugs were exhaustively extracted after 45 minutes when pH difference was used as the driving force and not voltage difference, i. e., conventional HF-LPME instead of EME. On the other hand all the drugs were exhaustively extracted after 10 minutes when EME was employed.

Analytical method

The analytical systems employed for separation and detection or quantification of the analytes in the 3-phase HF-LPME were also very variable. Differently from the 2-phase HF-LPME, GC was less used, and LC and CE were more employed again due to the aqueous nature of the acceptor phase used in this type of HF-LPME. Some of the studies were classified as 3-phase HF-LPME for presenting an acceptor phase different from the solvent within the walls of the fiber even though both are organic solvents [35, 79, 106, 139, 153, 163, 189, 190]. Daravani et al. [185] injected the aqueous extract directly onto the GC; according to the authors the water phase does not damage bonded and cross-linked nonpolar stationary phases, but a strong acid or base does. For that reason neutralization of the pH 2 aqueous extract by KOH was performed and glass wool was placed in the injector line to prevent non-volatile compounds originated from the neutralization reaction to reach the column. Some other studies [90, 92, 101, 102, 173, 191] dried the aqueous extract before reconstituting with an organic solvent to inject directly onto the instrument. All the studies that dried the samples and reconstituted them in organic solvent used derivatization in this process, except one [92].

Ref.	Analytes	Matri x	Donor phase	Fiber: Material Length id(mm)xwt(mm)xps(µm)	Solvent and Additives	Acceptor phase and Additives	Extraction process	Instrumentation
[107]	Methamphetamine	U P	2.5mL pH 13 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	HCl 0.1M pH 1	Stirring 400rpm 45min	CE-UV
[192]	Ibuprofen Naproxen Ketoprofen	U	2.5mL pH 1	PP 8cm 0.6x0.2x0.2	Dihexyl ether	NaOH 0.01mM pH 12	Vibrating 400rpm 45min	CE-UV
[44]	Metamphetamine (CE) Naproxen (CE) Citalopram (LC) NDCIT (LC)	U P	1-4mL Variable pH	PP 4 or 8cm 0.6x0.2x0.2	1-octanol	HCl 0.1M (CE) pH 1; NaOH 0.02M (LC) pH 12.3	Vibrating 1000rpm 45min	CE-UV LC-FD
[177]	Citalopram NDCIT	P	4mL pH *ca.*13	PP 8cm 0.6x0.2x0.2	Dihexyl ether	Phosphate buffer 20mM pH 2.75	Vibrating 1200rpm 60min	CE-UV
[85]	Methamphetamine Citalopram	U P B	4mL pH *ca.*13	PP 8cm 0.6x0.2x0.2 27cm 0.33x0.15x0.4	Dihexyl ether	HCl 0.1M pH 1	Vibrating 1500rpm 15min (U, P); 30min (B)	CE-UV
[86]	Amphetamines	B U	1 (B); 4 (U)mL pH *ca.* 13	PP 8cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.01M pH 2	Vibrating 1500rpm 15min	FIA-MS/MS
[193]	Mianserin	P	1mL pH *ca.* 13.5	PP 8cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.01M pH 2	Vibrating 1500rpm 45min	CE-UV
[48]	Methadone Promethazine Haloperidol	U P	4mL pH 13.1	PP 8cm 0.6x0.2x0.2	Dihexyl ether	HCl 10mM	Vibrating 1500rpm 45min	CE-UV
[166]	Citalopram Desmethylcitalopram	P	1.5mL pH *ca.* 13	PP 1.8cm 1.2x0.2x0.2	Dodecyl acetate	Phosphate 20mM pH 2.75	Vibrating 1500rpm 45min	CE-UV
[93]	Paroxetine Fluvoxamine Mianserin Citalopram	M	1.5mL pH *ca.* 13.5	PP 1.8cm 1.2x0.2x0.2	Polyphenyl-methylsiloxane	HCl 10mM pH 2	Vibrating 1500rpm 60min	CE-UV
[87]	Antidepressant drugs (TCA and SSRI)	P B	1.5mL pH 13.1	PP 1.8cm 1.2x0.2x0.2	Dodecyl acetate	Formic acid 200mM pH *ca.* 2	Vibrating 1500rpm 30min	LC-MS CE-UV
[39]	Amphetamine Morphine Practolol	P U	4mL pH 7 Sodium octanoate	PP 8.0cm 0.6x0.2x0.2	1-octanol	HCl 50mM pH 1.3	Vibrating 1500rpm 45min	CE-UV
[88]	Zolpidem Benzodiazepines	B	1.5mL pH 7.5	PP 1.8cm 1.2x0.2x0.2	Nonanol	HCl 0.4M pH 0.4	Vibrating 1500rpm 60min	LC-UV LC-MS
[167]	Amphetamines Pethidine Nortriptyline Methadone	P U	1mL pH *ca.* 13.5	PP 1.8cm 1.2x0.2x0.2	Plant fatty oils Plant essential oils	Formic acid 10mM pH 2.9	Vibrating 1200rpm 45min	CE-UV

	Haloperidol Loperamide							
[157]	Amphetamine Phenylpropanolamin e Cimetidine Morphine β-blockers	P	0.1mL pH 7 Sodium octanoate	PP 6.5cm 0.6x0.2x0.2	1-octanol	HCl 50mM pH 1.3	Vibrating 1500rpm 60min	LC-MS
[50]	Basic drugs	P	1.5mL pH 13 Sodium octanoate	PP 1.8cm 1.2x0.2x0.2	Dodecyl acetate	HCl 10mM pH 2	Vibrating 1500rpm 60min	CE-UV
[40]	Amphetamine Phenylpropanolamin e Metaraminol Cimetidine Morphine β-blockers	P	0.1mL pH 7 Bromothymol blue	PP 6.5cm 0.6x0.2x0.2	1-octanol or peppermint oil	HCl 50mM pH 1.3	Vibrating 1500rpm 60min	CE-UV
[51]	Steroids metabolite	U	n.r.	n.r.	Dihexyl ether	MSTFA:ammonium iodide:dithioerythritol (1000:2:4, v/m/m)	Stirring 1250rpm 45°C 30min	GC-MS
[194]	Imipramine Amitriptyline Setraline	P U	11mL pH 12	PP 8.8cm 0.6x0.2x0.2	1-dodecane	H_3PO_4 0.1M pH 2.1	Stirring 700rpm 30min	LC-UV
[108]	Clenbuterol	U	7.5mL pH 14 No salt added	PP 4.5cm 0.6x0.2x0.2	1-octanol	Formic acid 5M pH 1.5	Stirring 1000rpm 30min	LC-UV LC-MS/MS
[195]	Hydroxychloroquine and metabolites	U	*ca.* 4.3mL pH *ca.*13 10% (w/v) NaCl	PP 7cm 0.6x0.2x0.2	1-octanol	HCl 100mM pH 1	Stirring 1200rpm Room temperature (*ca.* 22°C) 40min	CE-UV
[97]	Chlorpromazine	U Se	11mL pH 11.8 No salt added	PP 8.8cm 0.6x0.2x0.2	1-dodecane	HCl 0.01M pH 2	Stirring 1000rpm Room temperature 20min	LC-UV
[109]	Strychnine Brucine	U	4mL pH *ca.*13.5 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	H_3PO_4 100mM pH 1.6	Stirring 1500rpm Room temperature 40min	CE-UV
[110]	Tetradrine Fangchinoline	P	4.5mL pH 8.5 No salt added	PP 7.5cm 0.6x0.2x0.2	1-octanol	HCl 5mM pH 2.3	Stirring 1100rpm Room temperature (*ca.* 22°C) 60 min	LC-UV
[174]	Mirtazapine and metabolites	P	4mL pH 8 15% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	Dihexyl ether	Acetic acid 0.01M pH 3.4	Sonicating *ca.* 35°C 45min	LC-MS
[111]	Mefloquine Carboxymefloquine	P	4mL pH *ca.*13.5 No salt added	PP 6.5cm 0.6x0.2x0.2	Dihexyl ether	$HClO_4$ 10mM pH 2	Stirring 1100rpm Room temperature (*ca.* 23°C)	LC-UV

							30 min	
[112]	Chloroquine and metabolites	P	4mL pH 11 No salt added	PP 7cm 0.6x0.2x0.2	1-octanol	TFA 0.1M	Stirring 1200rpm Room temperature (*ca.* 23°C) 30min	LC-MS/MS
[94]	Pethidine Nortriptyline Methadone Haloperidol Loperamide	P U M	1mL pH 2	PP 2.5cm 1.2x0.2x0.2	1-isopropyl-4-nitrobenzene	pH 2	Vibrating 1000rpm 10V 5min	CE-UV
[196]	TCA	P	1mL pH 10	PP 3.5cm 0.6x0.2x0.2	Dihexyl Ether	Sodium phosphate buffer 50mM pH 3	Stirring 400rpm 45min	CE-UV
[113]	Furosemide Bumetanide Triamterene	U	6mL pH 1.5 (for acidic) pH 12.5 (for basic) No salt added	PP 0.6x0.2x0.2	1-octanol	0.12M NaOH (for acidic) pH 13.1 0.04M H_3PO_4 (for basic) pH 1.9	Stirring 250rpm Room temperature (*ca.* 27°C) 50min	LC-UV
[111]	Mefloquine Carboxymefloquine	P	4mL pH *ca.* 12 then pH *ca.* 3	PP 15cm 0.6x0.2x0.2	Dihexyl ether	0.01M perchloric acid then 0.05M NaOH pH 2 and 12.7	Vibrating 1750rpm 30min	LC-UV
[89]	Pethidine Nortriptyline Tramadol Methadone Haloperidol Loperamide	P B	0.5mL	PP 2.5cm 1.2x0.2x0.2	1-ethyl-2-nitrobenzene	HCl 10mM	Vibrating 1050rpm 10V 10min	CE-UV
[197]	Ibuprofen	U	50mL pH 2	PP 27cm 0.6x0.2x0.2	Dihexyl ether	NaOH pH 10	Stirring 300rpm 15min	FIA-CL
[198]	Ibuprofen Diclofenac Salicylic acid	U	50mL pH 2	PP 27cm 0.6x0.2x0.2	Dihexyl ether	pH 12.5	Stirring 300rpm 15min	LC-UV LC-FD
[158]	Oxytetracycline Tetracycline Doxycycline	P	11mL pH *ca.* 9	PP 8.8cm 0.6x0.2x0.2	1-octanol + 10% (w/v) Aliquat-336	0.1M H_3PO_4 + 1M NaCl pH 1.6	Stirring 900rpm 35min	LC-UV
[199]	Pioglitazone	P U	10mL pH 8 10% (W/v) NaCl	PP 8.8cm 0.6x0.2x0.2	Dihexyl ether	HCl pH 2.2	Stirring 500rpm 30min	LC-UV
[114]	Rosiglitazone	P U	10mL pH 9.5 No salt added	PP 6cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.1M pH 1	Stirring 600rpm 30min	CE-UV LC-UV
[37]	Fluoxetine Norfluoxetine	P	5mL pH 14 No salt added	PP 7cm 0.6x0.2x0.2	Dihexyl ether	HCl 20mM pH 1.7	Stirring 1400rpm 40min	LC-FD
[115]	Gabapentin	P U	8.5mL No salt added FDNB	PP 8.8cm 0.6x0.2x0.2	Dihexyl ether	pH 9.1	Stirring 1250rpm Room temperature 45min	LC-UV
[168]	Amitriptyline Citalopram Fluoxetine	P	70μL pH *ca.* 7.4	PP 2.9cm 1.2x0.2x0.2	1-ethyl-2-nitrobenzene	HCOOH 10mM pH 2.9	No forced convection 9V 1min	LC-MS

	Fluvoxamine							
[36]	Ketoconazole Clotrimazole Miconazole	P U	10mL pH 11 NaCl 5% (w/v)	PP 8cm 0.6x0.2x0.2	Dihexyl ether	pH 2.5	Stirring 800rpm 45min	LC-UV
[184]	Amlodipine	P U	3mL pH 10	PP 8cm 0.6x0.2x0.2	NPOE	HCl 10mM pH 2	Stirring 1000rpm 200V 15min	CE-UV
[116]	Desipramine	P U	8mL pH 13 No salt added	PP 5cm 0.6x0.2x0.2	Propyl benzoate	HCl 1M pH 0	Stirring 700rpm Room temperature 15min	Voltametry
[117]	Phenazopyridine	P U	5mL pH 9 No salt added	PP 3.5cm 0.6x0.2x0.2	Diphenyl ether	H_2SO_4 0.1M pH 1	Stirring 1300rpm 45ºC 30min	FIA-DAD
[200]	Aristolochic acid	U	5mL pH 3	PP 3.3cm 0.6x0.2x0.2	1-octanol	NaOH 10mM pH 12	Stirring 800rpm 40min	LC-UV
[178]	Aconitine Hypaconitine Mesaconitine	U	5mL pH 11	PP 5.3cm 0.6x0.2x0.2	1-octanol	HCl 10mM pH 3	Stirring 800rpm 40ºC 60min	LC-UV
[118]	Matrine Sophocarpine	U	4mL pH 13.7 No salt added	PP 7cm 0.6x0.2x0.2	1-octanol	H_3PO_4 100mM pH 1.5	Stirring 600rpm 60min	LC-UV
[35]	Clotrimazole Miconazole	P U	24mL pH 8 No salt added	PP 8cm 0.6x0.2x0.2	1-dodecane	Acetonitrile	Stirring 900rpm 40min	GC-FID
[119]	Propylthiouracil	P U	7.5mL pH 12 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol + 6% (w/v) Aliquat 336	$NaClO_4$ 2M pH 9	Stirring 1250rpm 25ºC 40min	LC-UV
[120]	Dexamethasone	P U	7.5mL pH 3 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol + 5% (w/v) Aliquat 336	$NaClO_4$ 2M pH 9	Stirring 1250rpm Room temperature 60min	LC-UV
[121]	Desipramine	P U	8mL pH 13 No salt added	PP 5cm 0.6x0.2x0.2	Propyl benzoate	HCl 0.01M pH 2	Stirring 700rpm Room temperature 15min	Potentiometry
[122]	Mebendazole	P U	10mL pH 9 No salt added	PP 8.8cm 0.6x0.2x0.2	1-undecanol	HCl 100mM pH 1	Stirring 700rpm Room temperature 60min	LC-UV
[122]	Mebendazole	P U	7mL pH 1 No salt added	PP 8.8cm 0.6x0.2x0.2	NPOE	HCl 100mM pH 1	Stirring 700rpm Room temperature 150V 15min	LC-UV
[159]	Ephedrine	P U	7mL pH 11 NaCl 12% (w/v)	PP 7.5cm 0.6x0.2x0.2	Toluene + 10% (w/v) TEHP	HCl 1mM pH 3	Stirring 1200rpm Room temperature	LC-UV

							25min	
[159]	Ephedrine	P U	7mL pH 2	PP 7.5cm 0.6x0.2x0.2	NPOE + 10% (v/v) DEHP	HCl 100mM pH 1	Stirring 1000rpm Room temperature 100V 15min	LC-UV
[189]	Tramadol	P U	pH 11 Ionic strength 4M	PP 10cm 0.6x0.2x0.2	1-dodecane	Acetonitrile	Stirring 1000rpm 40min	GC-MS
[181]	Trimipramine Desipramine	P U	3mL pH *ca.* 12 5% (w/v) NaCl	PP 1.3cm 0.6x0.2x0.2	1-dodecane	Acetic acid 0.1M pH *ca.* 3	Stirring 860rpm 45ºC 20min	ESI-IMS
[160]	Naltrexone Nalmefene	P U	pH 2 pH *ca.* 10	PP 5.6cm 0.6x0.2x0.2	NPOE + DEHP (85:15 v/v)	HCl 100mM pH 1	Stirring 1250rpm 100V 20min	LC-UV
[123]	Pentazocine	P U	3mL pH 9 No salt added	PP 1.3cm 0.6x0.2x0.2	1-octanol	Acetic acid 0.5M pH *ca.* 3	Stirring 900rpm 20ºC 25min	ESI-IMS
[201]	Clomipramine	P U	3mL pH *ca.* 10 10% (w/v) NaCl	PP 3cm 0.6x0.2x0.2	1-dodecane	Methanol	Stirring 1700rpm 20min	CD-IMS
[124]	Alfentanil Fentanyl Sufentanil	P U	5mL pH *ca.* 10 No salt added	PP 3.5cm 0.6x0.2x0.2	Isoamyl benzoate	H_2SO_4 0.05M pH 1.3	Stirring 1200rpm 45ºC 20min	LC-UV
[125]	Amantadine	P U	3mL pH *ca.* 10 No salt added	PP 3cm 0.6x0.2x0.2	1-dodecane	Methanol	Stirring 1400rpm 20min	CD-IMS
[126]	Amphetamines	U	3mL pH 3 No salt added	PP 7cm 0.6x0.2x0.2	NPOE + 15% (v/v) TEHP	HCl 100mM pH 1	Stirring 1000rpm Room temperature 250V 7min	LC-UV
[127]	Thebaine	U	3mL pH 3 No salt added	PP 6cm 0.6x0.2x0.2	NPOE	HCl 100mM pH 1	Stirring 1250rpm 300V 15min	LC-UV
[99]	Atenolol Betaxolol Propranolol	OF	3mL pH 3 No salt added	PP 6cm 0.6x0.2x0.2	NPOE + 10% (v/v) DEHP + 5% (v/v) TEHP	HCl 100mM pH 1	Stirring 1250rpm 250V 15min	LC-UV
[100]	Levamisole	P U OF	4mL pH 2 No salt added	PP 9cm 0.6x0.2x0.2	NPOE + 5% (v/v) TEHP	HCl 100mM pH 1	Stirring 1000rpm 200V 15min	LC-UV
[169]	Atropine Scopolamine	P	pH 7.4	PP 10cm 0.55x0.45x0.18	1-heptanol:dimethyl benzene (30:70 v/v)	HCl 50mM pH 1.3	No forced convection 37ºC 5h	LC-UV
[128]	Nimesulide	P	5mL pH 2	PP 5.5cm	Dihexyl ether	NaOH 20mM pH 12.3	Stirring 400rpm	LC-UV

			No salt added				Room temperature (25ºC) 30min	
[129]	Bisoprolol	P	5.6mL pH *ca.* 14 No salt added	PVDF 8.5cm 0.6x0.2x0.2	1-octanol	Formic acid 1M pH 1.8	Stirring 800rpm 35ºC 25min	LC-FD
[185]	Imipramine Clomipramine	P U	2.1mL pH 4	PP 2.6cm 0.6x0.2x0.2	NPOE	pH 2	Stirring 1400rpm 200V 20min	GC-FID
[130]	Diclofenac	P U	2.1mL pH 11 No salt added	PP 3.1cm 1.2x0.2x0.2	1-octanol	NaOH 10mM pH 12	Stirring 1200rpm 30ºC 20 V 5min	LC-UV
[90]	Butalbital Secobarbital Pentobarbital Phenobarbital	B	1mL pH *ca.* 1	PP 9cm 0.6x0.2x0.2	Decanol	NaOH pH 13	Sonication 5min	GC-MS
[34]	AMPAs MPA	U	3mL pH 1 30% (w/v) NaCl	PP 3cm 0.6x0.2x0.2	1-octanol	NaOH pH 14	Stirring 600rpm 42ºC 50min	LC-MS
[38]	Methimazole	P U	7.5mL pH 12.2 CTAB 100mM No salt added	PP 8.8cm 0.6x0.2x0.2	Octanol	$NaClO_4$ 1.5M	Stirring 1250rpm 45ºC 50min	LC-UV
[180]	Chloropheniramine Dextromethorphan	P	7.5mL pH 12.5 2% (w/v) NaCl	PP 8.8cm 0.6x0.2x0.2	Hexadecane	HCl 0.5mM pH 3.3	Stirring 1250rpm 60min	LC-UV
[175]	Basic drugs	P	50µL pH 7.4	PP 3cm 0.6x0.2x0.2 3 fibers	NPOE	Formic acid 10mM pH 2.9	No forced convection 200V 10min	LC-MS
[131]	Ofloxacin Ciprofloxacin	P	10mL pH 8.5 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol + 10% (w/v) Aliquat 336	pH 1 1mM NaCl	Stirring 1000rpm 45min	LC-UV
[132]	Trimipamine	P U	5mL pH 4.5 No salt added	PP 8cm 0.6x0.2x0.2	NPOE	pH 1	Stirring 1000rpm 51V 34min	CE-UV
[133]	Amitriptyline Imipramine Trimipramine Clomipramine	P U	5mL pH 12 No salt added	PP 8cm 0.6x0.2x0.2	1-dodecane	Methanol + 0.01M HCl pH 2	Stirring 1000rpm 40min	LC-UV
[134]	Mitiglinide	P U	10mL pH 1.5 No salt added	PP 6cm 0.6x0.2x0.2	1-octanol	NaOH 0.1M pH 13	Stirring 300rpm Room temperature 45min	LC-UV
[135]	Warfarin	P	11mL pH 2.3 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol	0.1mM NaOH pH 11	Stirring 1000rpm 30min	LC-UV
[136]	Apigenin	U	11mL	PP	1-octanol	Carbonate 50mM	Stirring	LC-UV

			pH 3 No salt added	8.8cm 0.6x0.2x0.2		pH 11.5	1000rpm Room temperature 75min	
[137]	Amlodipine	P U	11mL pH 13 No salt added	PP 8.8cm 0.6x0.2x0.2	Dibenzyl ether	HCl 0.01M pH 2	Stirring 800rpm Room temperature 45min	LC-UV
[91]	Cathinone Amphetamines Ketamine DOI	B HB	80µL	PP 5cm 0.6x0.2x0.2	ENB	Acetic acid 10mM pH 3.4	No forced convection 15V 5min	LC-MS
[202]	Dextromethorphan Pseudoephedrine	P U	3mL pH 12.7 0% and 30% (w/v) NaCl for dextromethorphan and pseudoephedrine	PP 3cm 0.6x0.2x0.2	1-dodecane	Methanol	Stirring 750rpm 20min	CD-IMS
[138]	Hydroxyzine Cetirizine	P	10mL pH 5 → 11 No salt added	PP 8.2cm 0.6x0.2x0.2	1-octanol	pH 2	Stirring 1200rpm Room temperature 30min and then 20min	CE-UV
[101]	Amphetamines	H	50mg pH 14 1% (w/v) NaCl	PP 9cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.1M pH 1	Vibrating 1000rpm 45min	GC-MS
[139]	Desipramine	P U	3mL pH *ca.* 13 No salt added	PP 0.8cm 0.6x0.2x0.2	1-dodecanol	Methanol	Stirring 900rpm Room temperature 25min	GC-NPD
[140]	Nalmefene Diclofenac	U	24mL Neutral pH (6.5) No salt added	PP 3.8cm 0.6x0.2x0.2 2 fibers	NPOE + 5% (v/v) DEHP 1-octanol	HCl 50mM pH 1.3 NaOH 50mM pH 12.7	Stirring 700rpm 40V Room temperature 14min	LC-UV
[170]	Pethidine Diphenhydramine Nortriptyline Methadone	U	1mL pH 12.6	PP 20mm 1.2x0.2x0.2	1-octanol	HCl 10mM pH 2	Vibrating 1000rpm 30min	DESI-MS
[141]	Sufentanil	P U	4mL pH 2.5 No salt added	PP 8cm 0.6x0.2x0.2	NPOE	HCl 0.1M pH 1	Stirring 1000rpm 190V 28min	Voltametry
[161]	Dexamethasone	P U	7.5mL pH 6	PP 3.3cm 0.6x0.2x0.2	1-octanol + 5% (w/v) Aliquat 336	$NaClO_4$ 0.65 M pH 10	Stirring 500rpm 80min	LC-UV
[142]	Metformin	P U	10mL pH 13.4 PFBC 10mg No salt added	PP 4cm 0.6x0.2x0.2	Dihexyl ether	HCl 100mM pH 1	Stirring 300rpm 70°C 30min	LC-UV
[182]	NSAID	U	4mL pH 3 10% (w/v) NaCl	PP 4cm 0.6x0.2x0.2	Dihexyl ether	pH 13	Stirring 1500rpm 60°C 45min	LC-UV

[143]	Venlafaxine and metabolites	P	4mL pH 10 No salt added	PP 15cm 0.6x0.2x0.2	1-octanol	Acetic acid 0.1M pH *ca.* 3	Stirring 1750rpm 20min	LC-MS/MS
[186]	Tolterodine	P U	3mL pH 2	PP 8cm 0.6x0.2x0.2	NPOE	HCl 500 mM pH 0.3	Stirring 1200rpm 54V 20ºC 24min	CE-UV
[203]	Ketoprofen	P	5mL pH 2 5% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	1-octanol	pH 11	Stirring 600rpm Room temperature 30min	LC-UV
[155]	Trimetazidine	P	2.1mL pH 14 250mM sodium 1-octanesulfonate 7% (w/v) Na_2SO_4	PP 10cm 0.6x0.2x0.2	1-octanol	HCl 0.5M pH 0.3	Stirring 600rpm 25min	LC-UV
[102]	Butalbital Secobarbital Pentobarbital Phenobarbital	L	1mL pH 1.1	PP 9cm 0.6x0.2x0.2	Eucalyptus oil	NaOH 0.1M pH 13	Sonicating 5min	GC-MS
[156]	NSAID	U	pH 2	PP 13cm 0.6x0.2x0.2	Dihexyl ether	pH 12	Stirring 300rpm 20min	CE-UV
[156]	Sulfonamides	U	50mL pH 4 28% (w/v) Na_2SO_4	PP 27cm 0.6x0.2x0.2	1-octanol	pH 12	Stirring 300rpm 6h	LC-UV LC-FD
[162]	Morphine	U	4mL pH 6	PP 8cm 0.6x0.2x0.2	NPOE + 10% (v/v) TEHP + 10% (v/v) DEHP	HCl 0.1M pH 1	Stirring 1000rpm 90V 24min	DPV
[173]	Ketamine Norketamine Dehydronorketamine	U	pH 10 10% (w/v) NaCl	PP 7.5cm 0.6x0.2x0.2	Eucalyptus essential oil	HCl 1 M pH 0	Vibrating 2400rpm 30min	GC-MS
[171]	Pyrethroid and metabolites	U	1.2mL pH 4 Conc. HCl 8.3% (w/v) NaCl	PP 1cm 1.67x0.52x0.33	Dihexyl ether	NaOH 0.1M pH 13	120min	LC-UV
[92]	TCA Desmethylclomipram in Fluoxetine Norfluoxetine	B	4mL pH *ca.* 13	PP 8cm 0.6x0.2x0.2	Dodecane	Formic acid 0.1M pH 2.4	Stirring 1200rpm 55ºC 30min	GC-MS
[144]	Dextromethorphan	P U	4mL pH 6 No salt added	PP 8cm 0.6x0.2x0.2	NPOE	HCl 0.1M pH 1	Stirring 1000rpm 110V 20min	DPV
[145]	Pramipexole	P U	10mL pH 11.5 No salt added	PP 4cm 0.6x0.2x0.2	1-octanol	pH 3	Stirring 600rpm 25ºC 40min	LC-UV
[146]	Methamphetamine Cocaine	U	4mL pH 6.5	PP 5cm	NPOE + 10% (v/v) DEHP + 10% (v/v) TEHP	pH 1 pH 12	Stirring 1000rpm	CE-UV

	Methadone Buprenorphine Morphine Ibuprofen Ketoprofen Enalapril		No salt added	0.6x0.2x0.2 4 fibers	1-octanol 1-octanol + 4% (w/v) CTAB NPOE		50V 15min	
[68]	Atorvastatin	U	24mL pH 4 1.2% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-octanol	NaOH 0.001M pH 11	Stirring 1000rpm 60min	LC-UV
[147]	Carbegoline	P U	15mL pH 10 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	pH 3	Stirring 750rpm Room temperature (25°C) 30min	LC-UV
[176]	Aristolochic acid I Aristolochic acid II	P	4mL pH 3 2% (w/v) NaCl	PP 6cm 0.6x0.2x0.2	1-octanol	pH 11	Stirring (magnetofluid) 2000rpm Room temperature (25°C) 8min	LC-FD
[148]	Berberine Palmatine	P U	3mL pH 11.8 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	HCl 0.1M pH 1	Stirring 600rpm 25°C 10min	CE-UV
[187]	Propranolol	P U	4mL pH 3.5	PP 8cm 0.6x0.2x0.2	NPOE	pH 1	Stirring 1250rpm 40V 20°C 32min	CE-UV
[191]	THC-COOH	U	1mL pH < 3 1% (w/v) NaCl	PP 9cm 0.6x0.2x0.2	Dihexyl ether	NaOH 0.1mM pH 10	Shaking 1200rpm 30min	GC-MS
[190]	Cocaine Ketamine Lidocaine	U	14mL pH 11 20% (w/v) NaCl	PP 10cm 0.6x0.2x0.2	1-dodecane	Acetonitrile	Stirring 700rpm 30min	GC-MS
[98]	Lidocaine	Se U	5mL pH *ca.* 12 1% (w/v) KCl	PP 8.2cm 0.6x0.2x0.2	1-octanol	HCl 0.01M pH *ca.* 2	Stirring 1000rpm Room temperature 50min	LC-UV
[149]	Rosiglitazone Metformin	P U	10mL → 10.7mL pH 9 → *ca.* 14 No salt added No derivitizing agent → 10mg/mL PFBC (100uL)	4cm	Dihexyl ether	HCl 0.1M pH 1	Stirring 300rpm Room temperature → 70°C 30 min+30min	LC-UV
[150]	Citalopram	U	4mL pH 12.5 No salt added	PP 8.0cm 0.6x0.2x0.2	1-octanol	pH 2.2	Stirring 1000rpm 28min	CE-UV
[70]	Triamterene	U	24mL pH 14 11% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-decanol	pH 1	Stirring 800rpm 90min	LC-UV
[151]	Olanzapine Fluoxetine	U P	3mL pH 12 5% (w/v) NaCl	PP 2.8cm 1.2x0.2x0.2	1-octanol	HCl 25 or 10mM pH 1.6 or 2	Stirring 1000rpm 60min	LC-UV
[151]	Olanzapine Fluoxetine	U P	3mL pH 6	PP 2.8cm	NPPE	HCl 10mM pH 2	Stirring 1000rpm	LC-UV

			No salt added	1.2x0.2x0.2			200V 30min	
[179]	Benzodiazepines	U	2mL pH 10 10% (w/v) NaCl	PP 9cm 0.6x0.2x0.2	Dihexyl ether:1-nonanol (9:1 v/v)	HCl 3M pH 0	Vibrating 2400rpm 90min	GC-MS
[152]	Diclofenac Naproxen	U P	4mL pH 7.4 Triton X-100 0.2mM No salt added	PP 6cm 0.6x0.2x0.2	1-octanol	pH 12	Stirring 1000rpm 15V 15min	CE-UV
[188]	Phenazopyridine	U P	6.5mL Neutral pH	PP 7.5cm 0.6x0.2x0.2	NPOE	HCl 100mM pH 1	Stirring 1250rpm 100V 20min	LC-UV
[172]	Atrazine and degradation products	U	200mL pH 7 20% (w/v) NaCl	PP 20cm 0.28x0.05x0.1	Dihexyl ether	HCl 1M pH0	Stirring 150rpm Room temperature (*ca*. 20) 5h	LC-UV
[183]	Sitagliptin	U	15mL pH 10.5 35% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-octanol	pH 3	Stirring 1000rpm 25ºC 50min	LC-UV
[153]	Atorvastatin Lovastatin Simvastatin	U	18mL pH 2 No salt added	PP 10cm 0.6x0.2x0.2	1-dodecane + 5% (w/v) TOPO	Methanol + NaOH 0.1M pH 13	Stirring 1000rpm 45min	LC-UV GC-FID
[163]	Levonogestrel Megestrol	U	20mL No pH adjustment 10% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	1-dodecane + 5% (w/v) TOPO	Methanol	Stirring 1000rpm 40min	LC-UV
[204]	SSRI	P U	6mL pH 12.8 2% (w/v) NaCl	PP 3cm 0.6x0.2x0.2	Phenetole	Acetic acid 0.1M pH *ca.* 3	Stirring 1000rpm 40min	Sweeping- MEKC
[77]	Albendazole Triclabendazole	U	6mL pH 8 No salt added	PP 8.8cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Vibrating 3min	LC-FD
[95]	Codeine Naproxen Ketamine Ibuprofen	P M	4mL pH 6	PP 2.1cm 1.2x0.3x0.2	1-octanol (acidic) 2-ethyl hexanol (basic)		Stirring 750rpm 175V 25min	LC-UV
[95]	Codeine Naproxen Ketamine Ibuprofen	P M	4mL pH 6	PP 2.1cm 1.2x0.3x0.2	1-octanol (acidic) 2-ethyl hexanol (basic)	HCl 32mM pH 1.5 (basic) NaOH 32mM pH 12.5 (acidic)	Stirring 750rpm 150V 6min 400V 19min	LC-UV
[154]	Clozapine	P	30mL pH 4.5 No salt added	PP 10cm 1.2x0.3x0.2	NPOE	pH 4.5	Stirring 1000rpm 200V 18min	Voltametry
[164]	Nalidixic acid	U	5mL pH *ca.* 12	PP 10cm 0.6x0.2x0.2	1-octanol + Aliquat 336 (9:1 v/v)	NaCl 1M	Stirring 1000rpm *ca.* 25ºC 45min	LC-UV
[96]	Ketamine (B) Codenie (B)	P M	4mL pH 6	PP 2.1cm	2-ethyl hexane (B) 1-octanol (A)	HCl pH 1.5 (B) NaOH pH 12.5 (A)	Stirring 750rpm	LC-UV

	Naproxene (A) Ibuprofen (A)			1.2x0.3x0.2			150V 6min (A) → 400V 19min (B)	
[104]	Lamotrigine	P	4mL pH 9 No salt added	PP 15cm 0.6x0.2x0.2	1-octanol	HCl pH 4	Stirring 500rpm 30min Room T	CE-UV
[79]	Oxazepam Lorazepam	U P	25mL No pH adjustment	PP 7cm 0.6x0.2x0.2	n-dodecane + TOPO 7.5% (w/v)	Acetonitrile	Stirring 1000rpm 30min	LC-MS
[103]	Muscimol Tryptophan Tryptamine	U	10mL pH 4 No salt added	PP 8cm 0.6x0.2x0.2	Dihexyl ether + DEHPA 20% (w/w)	HCl 200mM	Stirring 800rpm 60min	LC-UV
[80]	Triamterene	U	24mL NaOH 3M 2M NaCl	PP 8.5cm 0.6x0.2x0.2	1-decanol	HCl pH 1	Stirring 800rpm 90min	LC-UV
[105]	Ibuprofen Diclofenac	U	4mL pH 10.5 No salt added	PP 6cm 0.6x0.2x0.2	1-octanol + 0.6% w/v C60 fullerene	NaOH pH 12.8	Stirring 1000rpm 28min 6V	LC-UV
[165]	Bismuth	P	5mL 5mM H_2SO_4	PP 3.5cm 0.6x0.2x0.2	1-octanol + 1% (v/v) DEHP	300mM H_2SO_4	Stirring 700rpm 10min 70V	UV-Vis
[106]	Diclofenac	U P	0.05M HCl No salt added	PP 2.5cm 0.6x0.2x0.2	n-dodecane	Methanol	Stirring 1000rpm 20min Room T	ESI-IMS

Table 3. 3-phase HF-LPME (and variants) of drugs of forensic interest in biological matrices. The concentration values of salt added were converted to % (w/v); the pH were calculated based on the concentration of base or acid in some cases. Abbreviations: (A) = acidic; Aliquat-336 = 3-caprylil methyl ammonium chloride; AMPAs = alkyl methylphosphonic acids; (B) = basic; B = whole blood; *ca*. = approximately; CE = capillary electrophoresis; DEHP = di-(2-ethylhexyl) phosphate; DOI = 2,5-dimethoxy-4-iodoamphetamine; FD = fluorescent detector; FDNB = 1-fluoro-2,4-dinitrobenzene; FIA = flow injection analysis; FID = flame ionization detector; GC = gas chromatography; H = hair; HB = haemolysed blood; id = internal diameter; L = liver; LC = high performance liquid chromatography; M = breast milk; MEKC = micellar electrokinetic chromatography; MPA = methylphosphonic acid; MS = mass spectrometry; MS/MS = tandem mass spectrometry; n.r. = not reported; Na_2SO_4 = sodium sulfate; NaCl = sodium chloride; NDCIT = N-desmethylcitalopram; NPD = nitrogen-phosphorus detector; NPOE = 2-nitrophenyl octylether; OF = oral fluid; P = plasma; PP = polypropylene; ps = pore size; PVDF = polyvinylidene difluoride; Se = serum; SSRI = selective serotonin reuptake inhibitors; T = temperature; THC-COOH = 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid; TCA = tricyclic antidepressants; TEHP = tris(2-ethylhexy)phosphate; TOPO = trioctylphosphine oxide; wt = wall thickness; U = urine; UV = ultra-violet; Vis = visible

5. CONCLUSIONS

All the research related to new extraction methods, and specifically to HF-LPME, places this technique as a powerful method during sample preparation in bioanalysis. The increasing number of published articles during the years shows how acceptable HF-LPME has become in laboratories that perform analyses of biological material.

The advantages of HF-LPME over traditional extraction methods bring several benefits to numerous fields of toxicology, including forensic toxicology, and should facilitate complex sample handling. This review highlights that researchers concluded that HF-LPME is simple, fast and versatile. Moreover, the method presents a green-chemistry approach with high selectivity and enrichment. In addition to these advantages, the presence of the fiber assures the absence of carryover and helps to filter the sample, resulting in clean extracts.

On the other hand, many articles cited the difficulty of dealing with small volumes of solvents and of extracting many drugs simultaneously.

HF-LPME clearly presents a high application potential for routine testing in analytical toxicology laboratories. It’s potential for automation and its versatility regarding the suitability to different matrices and analytes place the technique in the bright list of methods with high potential to be adopted in forensic toxicology laboratories. By opening the new perspectives in sample preparation, the HF-LPME offers promising results for the field.

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7. CONFLICTS OF INTEREST

Declarations of interest: none.

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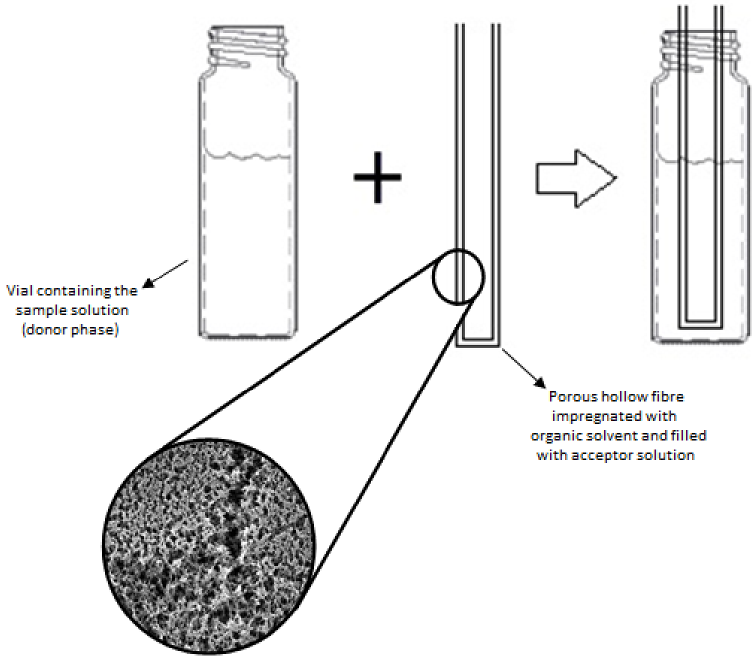

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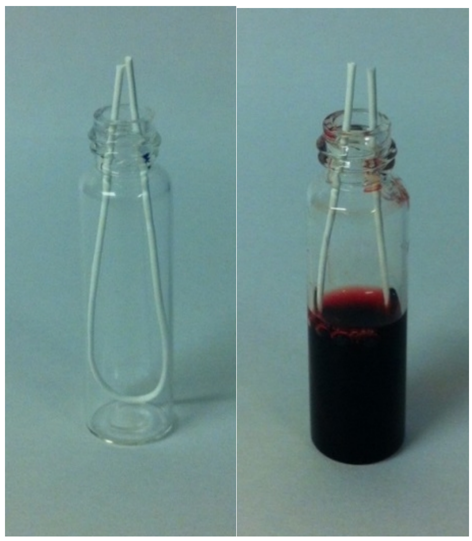


B

Figure 1. Schematic representation of the HF-LPME device (A) and its photo (B) – by courtesy of 3M.

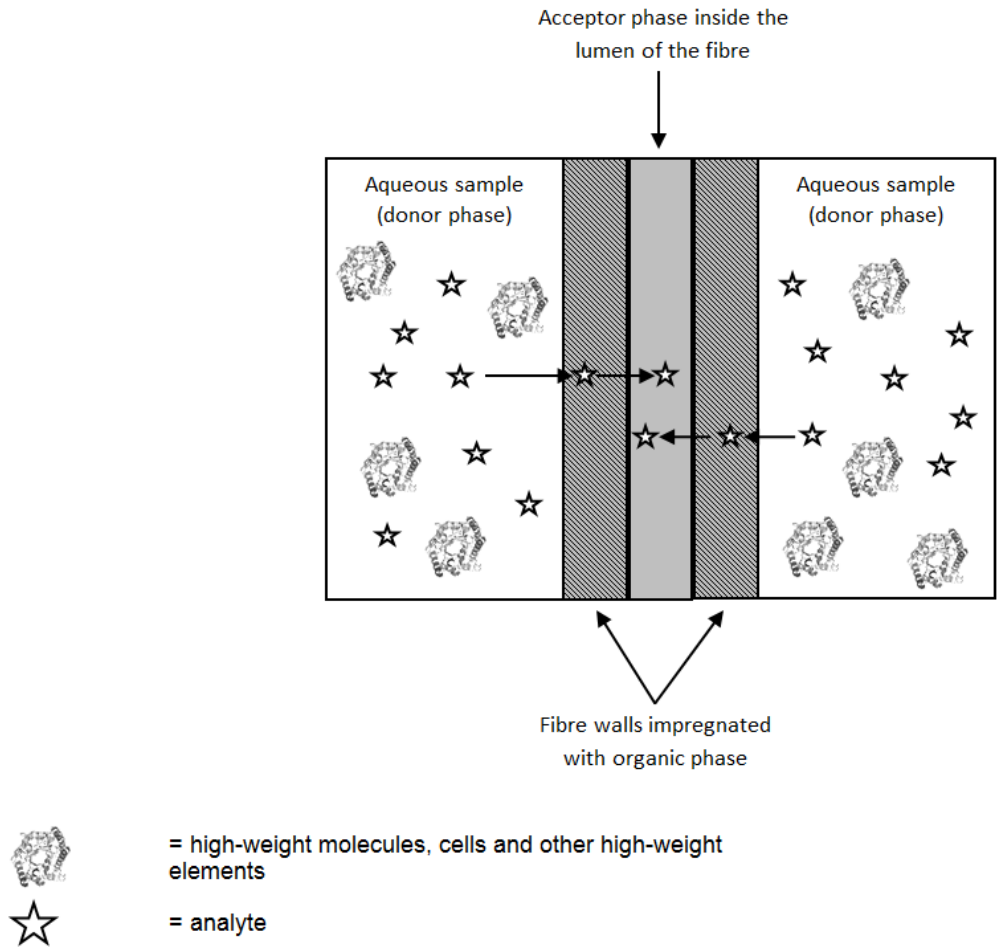


Figure 2. Schematic representation of the mechanisms by which HF-LPME works. In the 2-phase system, the lumen of the fiber is filled with the same organic solvent impregnated within the pores. In the 3-phase system, the lumen of the fiber is filled with a different solution responsible for accepting the analytes from the aqueous sample.

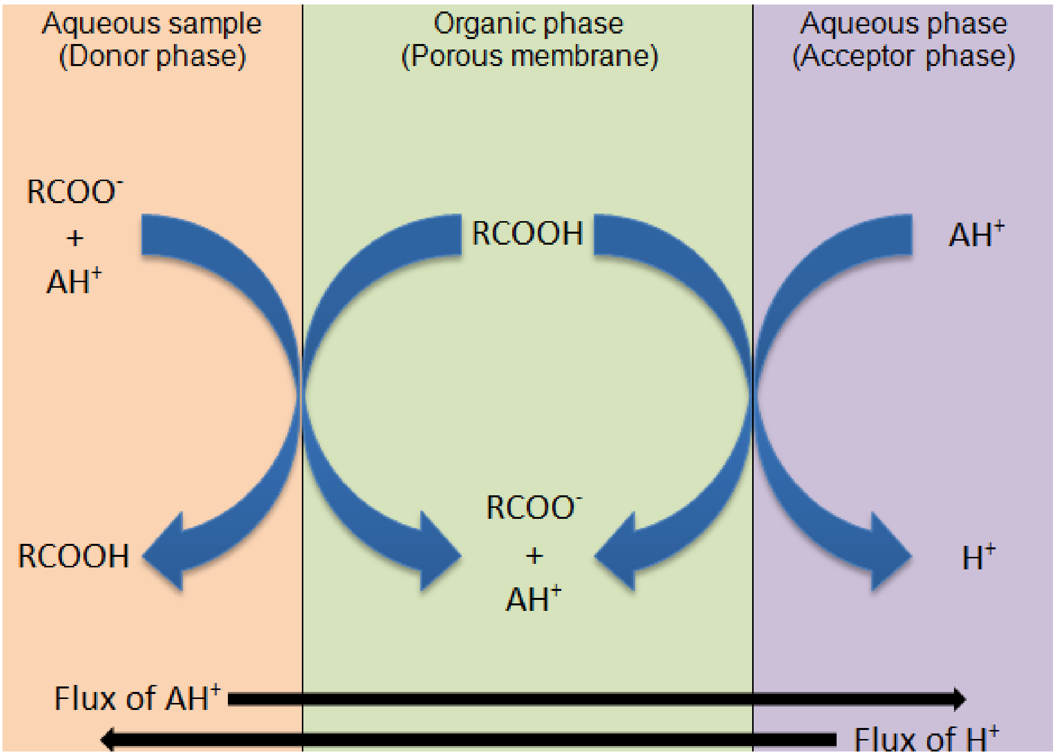


Figure 3. Schematic representation of carrier-mediated HF-LPME mechanism. AH$^+$ = ionized analyte; RCOO$^-$ = ionized carrier; RCOOH=non-ionized carrier. Adapted from [3, 39].

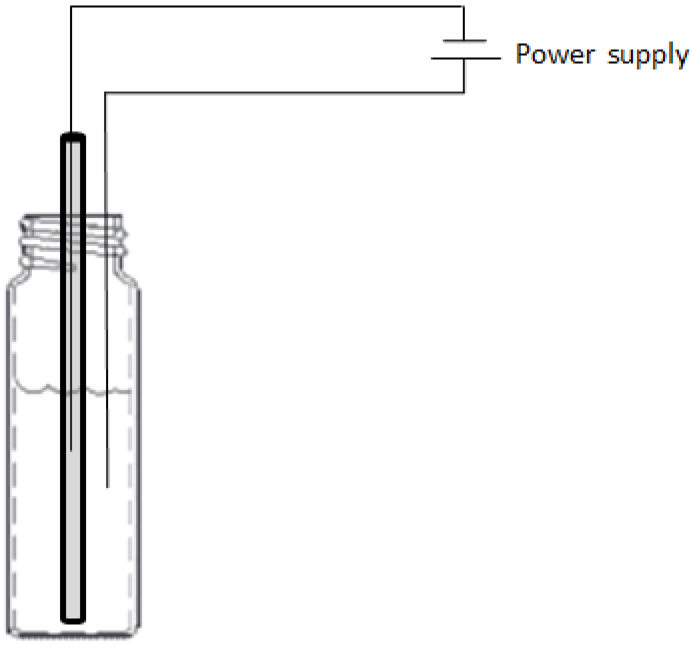


Figure 4. Schematic illustration of the equipment for EME

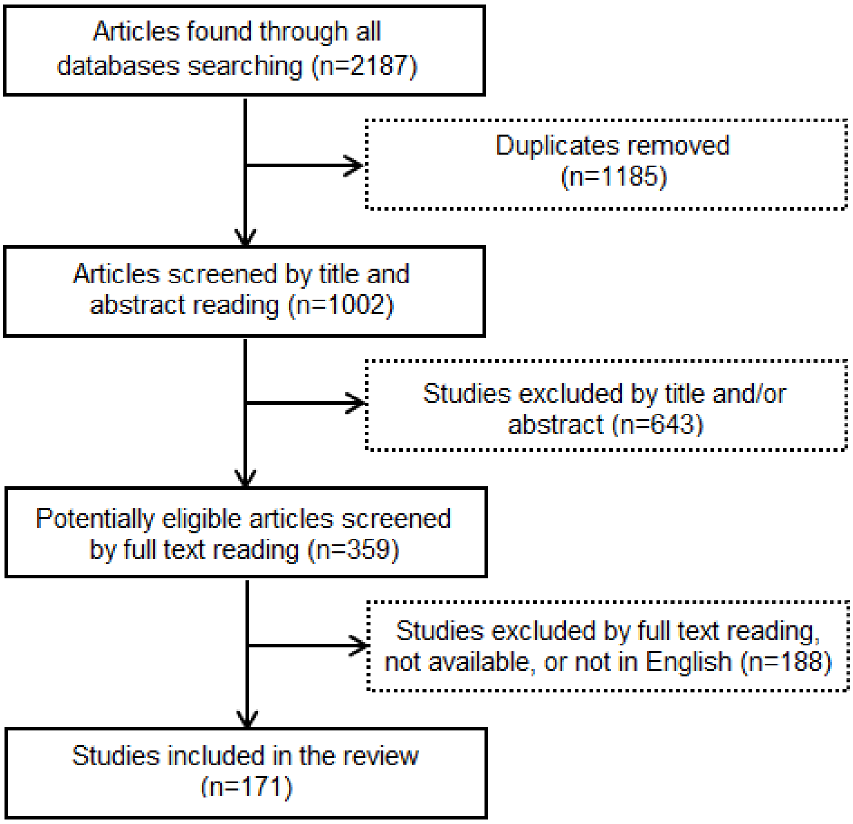
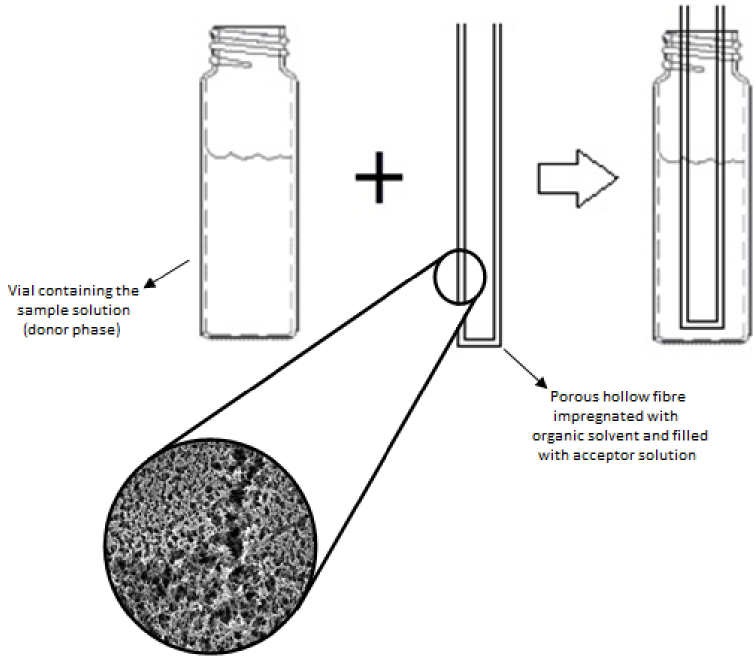
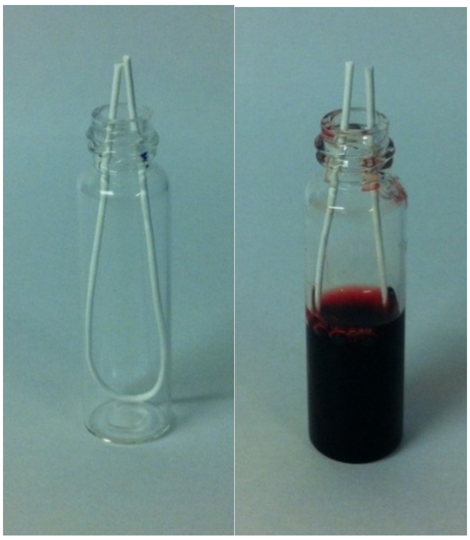


Figure 5. Flowchart of the studies selection



A



B

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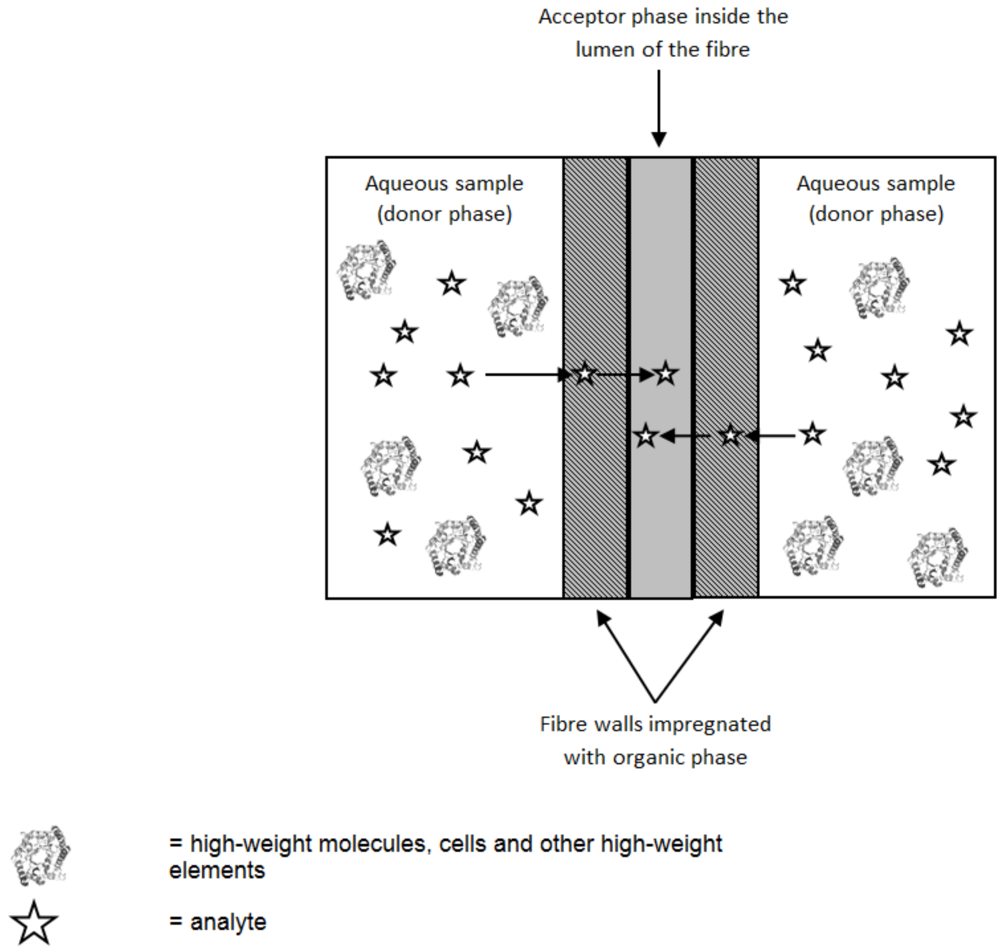

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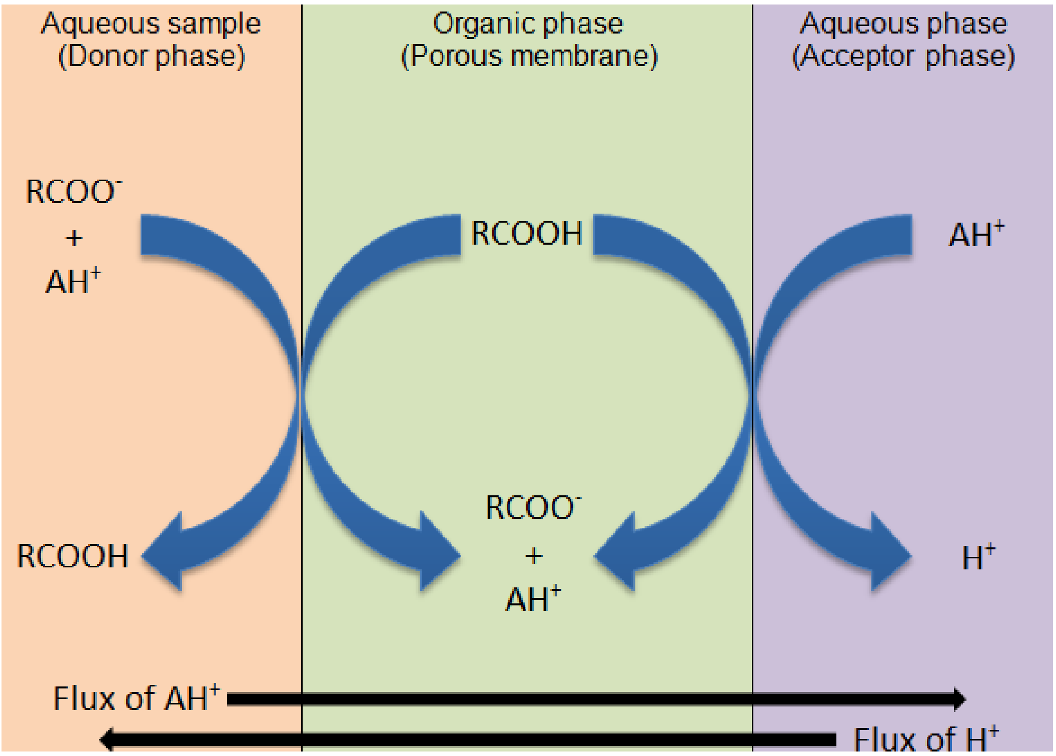

Figure 3. Schematic representation of carrier-mediated HF-LPME mechanism. AH^+ = ionized analyte; $RCOO^-$ = ionized carrier; RCOOH=non-ionized carrier. Adapted from [3, 39].

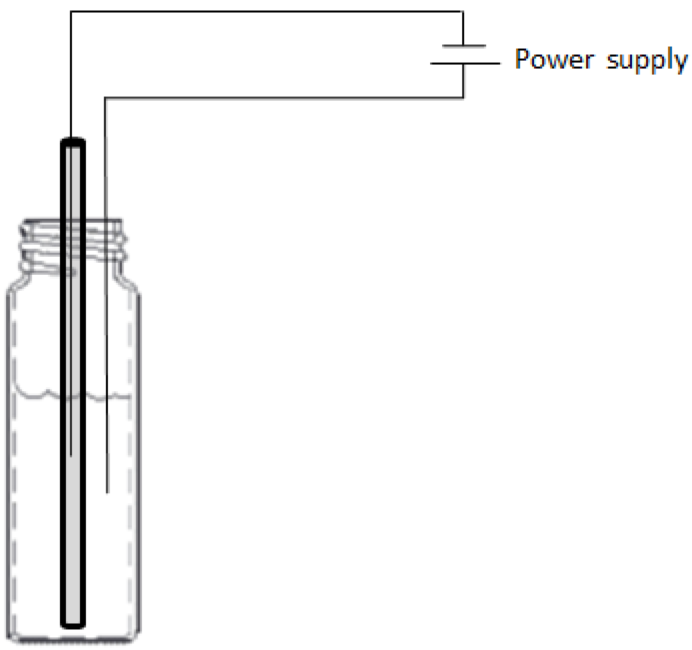

Figure 4. Schematic illustration of the equipment for EME

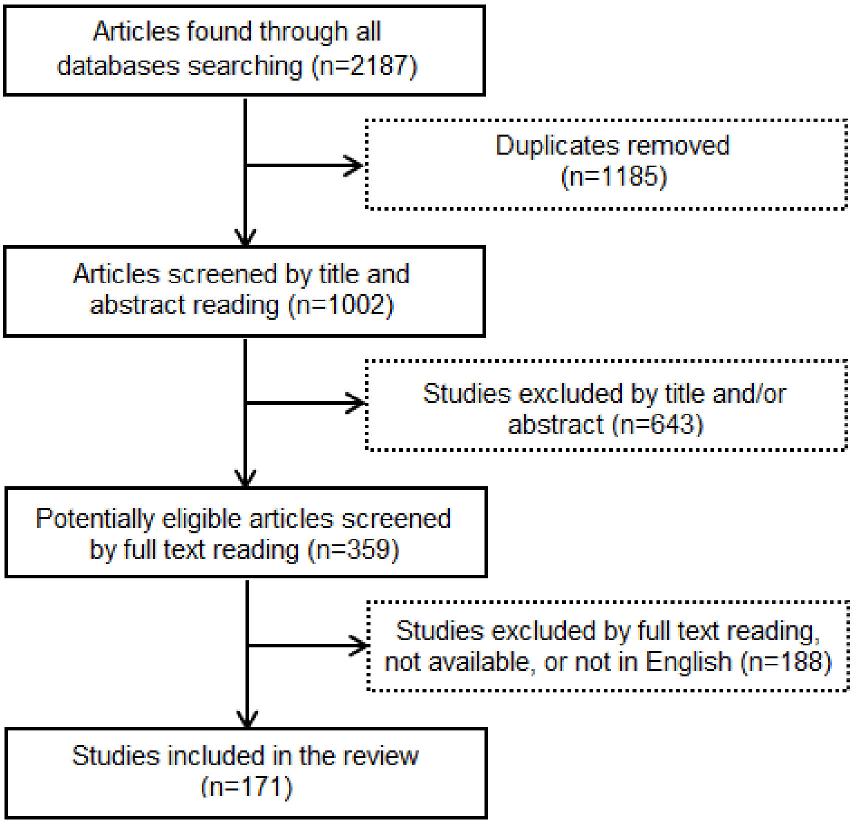

Figure 5. Flowchart of the studies selection

Criteria related to the publication	Language of publication is not English
	Article was not available as full-text
Criteria related to the extraction method	Not HF-LPME
	Dynamic HF-LPME
Criteria related to the purpose of the study	Review article
	Pharmacokinetic study
	Protein-binding investigation
	Octanol/water distribution investigation
Criteria related to the sample	Analysis of environmental samples (e.g. water, soil)
	Analysis of food (e.g. vegetables, milk)
	Biological sample not from human source (e.g. rat blood)
Criteria related to the analyte	Analysis of metals and related compounds (e.g. organometallics)
	Analysis of compounds from the environmental exposure (e.g. substances from pollution of air or water)
	Analysis of compounds from dietary exposure (e.g. nitrites, preservatives)
	Endogenous substances with no forensic interest (e.g. angiotensin, vitamins, hormones, non-exposure biomarkers)
	Other substances with no forensic interest (e.g. cosmetics)

Table 1. Exclusion criteria applied to the review.

Ref.	Analytes	Matrix	Donor phase: Volume pH Additives	Fiber: Material Length id(mm)xwt(mm)xps(μm)	Solvent and Additives	Acceptor phase and Additives	Extraction process	Instrumentation
[44]	Diazepam Prazepam	U P	1.5mL pH 5.5	PP 4cm 0.6x0.2x0.2	1-octanol	1-octanol	Vibrating 1000rpm 30min	GC-NPD
[45]	Diazepam NDMD	U P	3.5 (U); 3.0mL (P) pH 7.5 No salt added	PP 6cm 0.6x0.2x0.2	Butyl acetate:1-octanol (1:1 v/v) (U) Dihexyl ether:1-octanol (1:3 v/v) (P)	Butyl acetate:1-octanol (1:1 v/v) (U) Hexyl ether:1-octanol (1:3 v/v) (P)	Vibrating 600rpm 50min	GC-NPD
[46]	Cocaine Cocaethylene EMeE AEME	U	8mL pH 10.6	PP 6cm 0.6x0.2x0.2	Chloroform	Chloroform	Stirring 1600rpm 3min	GC-PDHID
[47]	THC-COOH	U	8mL pH 8 Bu_4N^+-HSO_4	PP 6cm 0.6x0.2x0.64	Octane:BSTFA (1:5 v/v)	Octane:BSTFA (1:5 v/v)	Stirring 1540rpm Room T 8min	GC-PDHID
[82]	Cocaine Cocaethylene EMeE AEME	OF	2.2mL pH 10.5	PP 7cm 0.6x0.2x0.2	Chlorophorm	Chlorophorm	Stirring 2000rpm 10min	GC-PDHID
[48]	Methadone Promethazine Haloperidol	U P	4mL pH 13.1	PP 8cm 0.6x0.2x0.2	Dihexyl ether	Dihexyl ether	Vibrating 1500rpm 45min	GC-FID
[49]	Mirtazapine	P	4mL pH 13.6 No salt added	PP 7cm 0.6x0.2x0.2	Toluene	Toluene	Stirring 30min *ca.* 22°C	LC-UV
[50]	Basic drugs	P	pH 7	PP 6.5cm 0.6x0.2x0.2	1-octanol	1-octanol	Vibrating 1500rpm 60min	CE-UV
[51]	Anabolic steroids	U	4mL pH 7 No salt added	PP 6cm 0.6xn.r.x0.2	1-octanol	1-octanol	Stirring 1250rpm Room T 45min	LC-MS
[52]	Thiazide diuretics Clopamide Probenecid Loop diuretics	U	7.5mL pH 2 15% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 1010rpm 40°C 40min	LC-MS/MS
[53]	Flunitrazepam	P U	4mL pH 9.5 (U); 8.0 (P) No salt added	PVDF 1.8cm 1.2x0.2x0.2	p-xylene (U) p-xylene:1-octanol (3:7 v/v) (P)	p-xylene (U) p-xylene/1-octanol (3:7 v/v) (P)	Stirring 375 (P); 450 (U) rpm 30°C 30min	GC-MS/MS
[54]	Clenbuterol Metoprolol Propranolol	U	5mL pH 12 14% (w/v) NaCl	PP 1.6cm 0.6x0.2x0.2	Methylbenzol	Methylbenzol:MSTFA (1:1 v/v)	Stirring 925rpm 35°C 20min	GC-MS
[75]	Free cyanide	U OF	5mL pH 6.5 Saturated with Na_2SO_4	Polyethersulfone 1.5cm 3.75x0.75x0.2	Sodium carbonate + Ni(II)-NH_3	Sodium carbonate + Ni(II)-NH_3 pH 11	Stirring 900rpm Room T 10min	CE-UV
[83]	THC CBD CBN	H	10mg pH 14 6.8% (w/v) NaCl	PP 6cm 0.6x0.2x0.2	Butyl acetate	Butyl acetate	Stirring 600rpm Room T 20min	GC-MS/MS

[55]	Promethazine Promazine Chlorpromazine Trifluoperazine	U	3mL pH 9 No salt added	PP 1.5cm 0.6x0.2x0.2	Toluene	Toluene	Stirring 1000rpm 40°C 10min	GC-FPD GC-FID
[5]	Amphetamines Caffeine Ketamine	U	3mL pH 12.5 30% (w/v) NaCl	PP 1cm 0.6x0.2x0.2	o-xylene	o-xylene	Stirring 1000rpm Room T (30°C) 20min	GC-FID
[56]	Pyrethroid metabolites	U	5mL pH *ca.* 1	PP 1.5, 2.0, 2.5 and 3.0cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 70°C 10min	GC-ECD
[57]	Alfentanil Fentanyl Sufentanil	P U	3mL pH *ca.* 11 No salt added	PP 1cm 0.6x0.2x0.2	Dihexyl acetate	Dihexyl acetate	Stirring 1000rpm Room T (25°C) 15min	GC-NPD
[81]	Amitriptyline Imipramine Promethazine	B	30µL pH 11 No salt added	PP 1cm 0.6x0.2x0.2	Toluene	Toluene	Stirring Room T 10min	GC-MS
[58]	Tramadol	P U	12mL pH 12 No salt added	PP 1.5cm n.r.	1-nonanol	1-nonanol	Stirring 1000rpm Room T 25min	GC-MS
[59]	Guaifenesin	P	25mL pH 7.4 1.7% (w/v) K_2HPO_4	PP 8cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 600rpm 37°C 30min	LC-FD
[60]	Anabolic steroids	U H	20mL No pH adjustment 7.5% (w/v) NaCl	PP 1.2cm 1.8x0.2x0.2	Toluene	Toluene	Stirring 750rpm 40°C 30min	GC-MS
[61]	Imipramine Desipramine Citalopram Sertraline	U	1.2mL Neutral (pH *ca.* 7)	PP 2.2cm 0.6x0.2x0.2	1-heptanol	1-heptanol	Stirring 1400rpm 60V 15min	GC-MS
[62]	Sulfetanil Alfentanil	P U	5mL pH 10 15% (w/v) NaCl	PP 1.3cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 700rpm 50°C 25min	GC-FID
[63]	Fluoxetine Norfluoxetine	P	5mL pH 11 No salt added	PP 3.7cm 0.6x0.2x0.2	Dihexyl ether	Dihexyl ether	Vibrating 700rpm 30min	GC-MS
[64]	Benzodiazepines	P U	*ca.* 5 (P); *ca.* 25 (U) mL pH *ca.* 9 No salt added	PP 10cm 0.6x0.2x0.2	Supramolecular solvent	Supramolecular solvent	Stirring 900rpm 50min	LC-UV
[65]	Indomethacin Dexamethasone Propafenone	P U	1.8ml pH 2, 2-8, 10 20% (w/v) NaCl	PVDF 3.5cm n.r.xn.r.xn.r.	1-octanol	1-octanol	Vibrating 173rpm Room T 102, 120 and 102min	LC-UV
[66]	Methadone	P U	10mL pH 11.5 5% (w/v) NaCl	PP 2cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Stirring 700rpm 20°C 45min	GC-FID
[67]	Nicotine	P	4.5mL pH 7.4 29% (w/v) NaCl	n.r. 3cm n.r.	1-octanol	1-octanol	Sonicating 37°C 10min	GC-FID

[68]	Amlodipine	U	24mL pH 10 1.2% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 1000rpm 60min	LC-UV
[69]	Naloxone Buprenorphine Norbuprenorphine	P	5mL pH 8.7 No salt added	PVDF 4cm 0.8x0.175x0.16	1-octanol:chlorophorm:toluene (2:4:4 v/v/v)	1-octanol:chlorophorm:toluene (2:4:4 v/v/v)	Stirring 1000rpm 20°C 30min	ULC-MS
[70]	Hydrochlorothiazide	U	24mL pH 12 No salt added	PP 8.5cm 0.6x0.2x0.2	1-octanol + 2% (w/v) Aliquat 336	1-octanol + 2% (w/v) Aliquat 336	Stirring 800rpm 90min	LC-UV
[71]	Amphetamines Methcatinone Ketamine Meperidine Methadone	U B	8mL pH 13	PP 4cm 0.6x0.2x0.2	Toluene	Toluene	Stirring 500rpm 30°C 15min	GC-MS
[72]	Flunitrazepam	P U	4mL pH 9.5 (U); 8.0 (P) No salt added	PVDF 1.8cm 1.2x0.2x0.2	p-xylene (U) p-xylene:1-octanol (3:7 v/v) (P)	p-xylene (U) p-xylene:1-octanol (3:7 v/v) (P)	Stirring 375 (P); 450 (U) rpm 30°C 30min	GC-MS
[73]	Metamphetamine	H U	4mL pH 7 No salt added	PP 6cm 0.6x0.2x0.2	1-octanol + 2.5mg/mL grapheme oxide	1-octanol	Stirring 1000rpm 60V 20min	GC-FID
[74]	Memantine	P	10mL pH 13 No salt added	PP 8cm 0.6x0.2x0.2	Cyclohexane	Cyclohexane + 0.3mg/mL dansyl chloride + 4% (v/v) triethylamine + 10% (v/v) acetone	Stirring 800rpm 40°C 50min	LC-FD
[76]	Naproxen Nabumetone	P U	pH 3 KCl 4% (w/v)	PP 4cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Stirring 600rpm 45°C 20min	LC-FD
[77]	Albendazole Triclabendazole	U	pH 8 No salt added	PP 8.8cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Vortexing Room T 3min	LC-FD
[79]	Oxazepam Lorazepam	U P	25mL No pH adjustment 7.5% (w/v) NaCl	PP 10cm 0.6x0.2x0.2	1-octanol	1-octanol	Stirring 1000rpm 50min	LC-MS
[80]	HCTZ	U	24mL pH 12 No salt added	PP 8.5cm 0.6x0.2x0.2	1-octanol + Aliquat 336 2% (w/w)	1-octanol + Aliquat 336 2% (w/w)	Stirring 800rpm 90min	LC-UV
[78]	Warfarin	P	8mL pH 6.5 No salt added	PP 3cm 0.6x0.2x0.2	1-octanol + CTAB 10mM	1-octanol + CTAB 10mM	Stirring 800rpm Room T 25min	UV-Vis

Table 2. 2-phase HF-LPME (conventional and variants) of drugs of forensic interest in biological matrices. The concentration values of salt added were converted to % (w/v); the pH were calculated based on the concentration of base or acid in some cases. Abbreviations: (A) = acidic; AEME = anhydroecgonine methyl ester; Aliquat-336 = 3-caprylil methyl ammonium chloride; (B) = basic; B = whole blood; BSTFA = bis(trimethylsilyl)trifluoroacetamide; Bu_4N^+-HSO_4= tetra-n-butylammonium; *ca*. = approximately; CBD = cannabidiol; CBN = cannabinol; CE = capillary electrophoresis; ECD = electron capture detector; EMeE = ecgonine methyl ester; FD = fluorescent detector; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; H = hair; id = internal diameter; K_2HPO_4 = dipotassium phosphate; LC = high performance liquid chromatography; MS = mass spectrometry; MS/MS = tandem mass spectrometry; n.r. = not reported; Na_2SO_4 = sodium sulfate; NaCl = sodium chloride; NDMD = N-desmethyldiazepam; NPD = nitrogen-phosphorus detector; OF = oral fluid; P

= plasma; PDHID = pulsed-discharge helium ionization detector-helium ionization detector; PP = polypropylene; ps = pore size; PVDF = polyvinylidene difluoride; T = temperature; THC-COOH = 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid; THC = Δ9-tetrahydrocannabinol; wt = wall thickness; U = urine; ULC = ultra-high performance liquid chromatography; UV = ultra-violet

Ref.	Analytes	Matri x	Donor phase	Fiber: Material Length id(mm)xwt(mm)xps(µm)	Solvent and Additives	Acceptor phase and Additives	Extraction process	Instrumentation
[107]	Methamphetamine	U P	2.5mL pH 13 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	HCl 0.1M pH 1	Stirring 400rpm 45min	CE-UV
[192]	Ibuprofen Naproxen Ketoprofen	U	2.5mL pH 1	PP 8cm 0.6x0.2x0.2	Dihexyl ether	NaOH 0.01mM pH 12	Vibrating 400rpm 45min	CE-UV
[44]	Metamphetamine (CE) Naproxen (CE) Citalopram (LC) NDCIT (LC)	U P	1-4mL Variable pH	PP 4 or 8cm 0.6x0.2x0.2	1-octanol	HCl 0.1M (CE) pH 1; NaOH 0.02M (LC) pH 12.3	Vibrating 1000rpm 45min	CE-UV LC-FD
[177]	Citalopram NDCIT	P	4mL pH *ca.*13	PP 8cm 0.6x0.2x0.2	Dihexyl ether	Phosphate buffer 20mM pH 2.75	Vibrating 1200rpm 60min	CE-UV
[85]	Methamphetamine Citalopram	U P B	4mL pH *ca.*13	PP 8cm 0.6x0.2x0.2 27cm 0.33x0.15x0.4	Dihexyl ether	HCl 0.1M pH 1	Vibrating 1500rpm 15min (U, P); 30min (B)	CE-UV
[86]	Amphetamines	B U	1 (B); 4 (U)mL pH *ca.* 13	PP 8cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.01M pH 2	Vibrating 1500rpm 15min	FIA-MS/MS
[193]	Mianserin	P	1mL pH *ca.* 13.5	PP 8cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.01M pH 2	Vibrating 1500rpm 45min	CE-UV
[48]	Methadone Promethazine Haloperidol	U P	4mL pH 13.1	PP 8cm 0.6x0.2x0.2	Dihexyl ether	HCl 10mM	Vibrating 1500rpm 45min	CE-UV
[166]	Citalopram Desmethylcitalopram	P	1.5mL pH *ca.* 13	PP 1.8cm 1.2x0.2x0.2	Dodecyl acetate	Phosphate 20mM pH 2.75	Vibrating 1500rpm 45min	CE-UV
[93]	Paroxetine Fluvoxamine Mianserin Citalopram	M	1.5mL pH *ca.* 13.5	PP 1.8cm 1.2x0.2x0.2	Polyphenyl-methylsiloxane	HCl 10mM pH 2	Vibrating 1500rpm 60min	CE-UV
[87]	Antidepressant drugs (TCA and SSRI)	P B	1.5mL pH 13.1	PP 1.8cm 1.2x0.2x0.2	Dodecyl acetate	Formic acid 200mM pH *ca.* 2	Vibrating 1500rpm 30min	LC-MS CE-UV
[39]	Amphetamine Morphine Practolol	P U	4mL pH 7 Sodium octanoate	PP 8.0cm 0.6x0.2x0.2	1-octanol	HCl 50mM pH 1.3	Vibrating 1500rpm 45min	CE-UV
[88]	Zolpidem Benzodiazepines	B	1.5mL pH 7.5	PP 1.8cm 1.2x0.2x0.2	Nonanol	HCl 0.4M pH 0.4	Vibrating 1500rpm 60min	LC-UV LC-MS
[167]	Amphetamines Pethidine Nortriptyline Methadone	P U	1mL pH *ca.* 13.5	PP 1.8cm 1.2x0.2x0.2	Plant fatty oils Plant essential oils	Formic acid 10mM pH 2.9	Vibrating 1200rpm 45min	CE-UV

	Haloperidol Loperamide							
[157]	Amphetamine Phenylpropanolamin e Cimetidine Morphine β-blockers	P	0.1mL pH 7 Sodium octanoate	PP 6.5cm 0.6x0.2x0.2	1-octanol	HCl 50mM pH 1.3	Vibrating 1500rpm 60min	LC-MS
[50]	Basic drugs	P	1.5mL pH 13 Sodium octanoate	PP 1.8cm 1.2x0.2x0.2	Dodecyl acetate	HCl 10mM pH 2	Vibrating 1500rpm 60min	CE-UV
[40]	Amphetamine Phenylpropanolamin e Metaraminol Cimetidine Morphine β-blockers	P	0.1mL pH 7 Bromothymol blue	PP 6.5cm 0.6x0.2x0.2	1-octanol or peppermint oil	HCl 50mM pH 1.3	Vibrating 1500rpm 60min	CE-UV
[51]	Steroids metabolite	U	n.r.	n.r.	Dihexyl ether	MSTFA:ammonium iodide:dithioerythritol (1000:2:4, v/m/m)	Stirring 1250rpm 45^{o}C 30min	GC-MS
[194]	Imipramine Amitriptyline Setraline	P U	11mL pH 12	PP 8.8cm 0.6x0.2x0.2	1-dodecane	H_3PO_4 0.1M pH 2.1	Stirring 700rpm 30min	LC-UV
[108]	Clenbuterol	U	7.5mL pH 14 No salt added	PP 4.5cm 0.6x0.2x0.2	1-octanol	Formic acid 5M pH 1.5	Stirring 1000rpm 30min	LC-UV LC-MS/MS
[195]	Hydroxychloroquine and metabolites	U	*ca.* 4.3mL pH *ca.*13 10% (w/v) NaCl	PP 7cm 0.6x0.2x0.2	1-octanol	HCl 100mM pH 1	Stirring 1200rpm Room temperature (*ca.* 22^{o}C) 40min	CE-UV
[97]	Chlorpromazine	U Se	11mL pH 11.8 No salt added	PP 8.8cm 0.6x0.2x0.2	1-dodecane	HCl 0.01M pH 2	Stirring 1000rpm Room temperature 20min	LC-UV
[109]	Strychnine Brucine	U	4mL pH *ca.*13.5 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	H_3PO_4 100mM pH 1.6	Stirring 1500rpm Room temperature 40min	CE-UV
[110]	Tetradrine Fangchinoline	P	4.5mL pH 8.5 No salt added	PP 7.5cm 0.6x0.2x0.2	1-octanol	HCl 5mM pH 2.3	Stirring 1100rpm Room temperature (*ca.* 22^{o}C) 60 min	LC-UV
[174]	Mirtazapine and metabolites	P	4mL pH 8 15% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	Dihexyl ether	Acetic acid 0.01M pH 3.4	Sonicating *ca.* 35^{o}C 45min	LC-MS
[111]	Mefloquine Carboxymefloquine	P	4mL pH *ca.*13.5 No salt added	PP 6.5cm 0.6x0.2x0.2	Dihexyl ether	$HClO_4$ 10mM pH 2	Stirring 1100rpm Room temperature (*ca.* 23^{o}C)	LC-UV

							30 min	
[112]	Chloroquine and metabolites	P	4mL pH 11 No salt added	PP 7cm 0.6x0.2x0.2	1-octanol	TFA 0.1M	Stirring 1200rpm Room temperature (*ca.* 23^{o}C) 30min	LC-MS/MS
[94]	Pethidine Nortriptyline Methadone Haloperidol Loperamide	P U M	1mL pH 2	PP 2.5cm 1.2x0.2x0.2	1-isopropyl-4-nitrobenzene	pH 2	Vibrating 1000rpm 10V 5min	CE-UV
[196]	TCA	P	1mL pH 10	PP 3.5cm 0.6x0.2x0.2	Dihexyl Ether	Sodium phosphate buffer 50mM pH 3	Stirring 400rpm 45min	CE-UV
[113]	Furosemide Bumetanide Triamterene	U	6mL pH 1.5 (for acidic) pH 12.5 (for basic) No salt added	PP 0.6x0.2x0.2	1-octanol	0.12M NaOH (for acidic) pH 13.1 0.04M H_3PO_4 (for basic) pH 1.9	Stirring 250rpm Room temperature (*ca.* 27^{o}C) 50min	LC-UV
[111]	Mefloquine Carboxymefloquine	P	4mL pH *ca.* 12 then pH *ca.* 3	PP 15cm 0.6x0.2x0.2	Dihexyl ether	0.01M perchloric acid then 0.05M NaOH pH 2 and 12.7	Vibrating 1750rpm 30min	LC-UV
[89]	Pethidine Nortriptyline Tramadol Methadone Haloperidol Loperamide	P B	0.5mL	PP 2.5cm 1.2x0.2x0.2	1-ethyl-2-nitrobenzene	HCl 10mM	Vibrating 1050rpm 10V 10min	CE-UV
[197]	Ibuprofen	U	50mL pH 2	PP 27cm 0.6x0.2x0.2	Dihexyl ether	NaOH pH 10	Stirring 300rpm 15min	FIA-CL
[198]	Ibuprofen Diclofenac Salicylic acid	U	50mL pH 2	PP 27cm 0.6x0.2x0.2	Dihexyl ether	pH 12.5	Stirring 300rpm 15min	LC-UV LC-FD
[158]	Oxytetracycline Tetracycline Doxycycline	P	11mL pH *ca.* 9	PP 8.8cm 0.6x0.2x0.2	1-octanol + 10% (w/v) Aliquat-336	0.1M H_3PO_4 + 1M NaCl pH 1.6	Stirring 900rpm 35min	LC-UV
[199]	Pioglitazone	P U	10mL pH 8 10% (W/v) NaCl	PP 8.8cm 0.6x0.2x0.2	Dihexyl ether	HCl pH 2.2	Stirring 500rpm 30min	LC-UV
[114]	Rosiglitazone	P U	10mL pH 9.5 No salt added	PP 6cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.1M pH 1	Stirring 600rpm 30min	CE-UV LC-UV
[37]	Fluoxetine Norfluoxetine	P	5mL pH 14 No salt added	PP 7cm 0.6x0.2x0.2	Dihexyl ether	HCl 20mM pH 1.7	Stirring 1400rpm 40min	LC-FD
[115]	Gabapentin	P U	8.5mL No salt added FDNB	PP 8.8cm 0.6x0.2x0.2	Dihexyl ether	pH 9.1	Stirring 1250rpm Room temperature 45min	LC-UV
[168]	Amitriptyline Citalopram Fluoxetine	P	70µL pH *ca.* 7.4	PP 2.9cm 1.2x0.2x0.2	1-ethyl-2-nitrobenzene	HCOOH 10mM pH 2.9	No forced convection 9V 1min	LC-MS

	Fluvoxamine							
[36]	Ketoconazole Clotrimazole Miconazole	P U	10mL pH 11 NaCl 5% (w/v)	PP 8cm 0.6x0.2x0.2	Dihexyl ether	pH 2.5	Stirring 800rpm 45min	LC-UV
[184]	Amlodipine	P U	3mL pH 10	PP 8cm 0.6x0.2x0.2	NPOE	HCl 10mM pH 2	Stirring 1000rpm 200V 15min	CE-UV
[116]	Desipramine	P U	8mL pH 13 No salt added	PP 5cm 0.6x0.2x0.2	Propyl benzoate	HCl 1M pH 0	Stirring 700rpm Room temperature 15min	Voltametry
[117]	Phenazopyridine	P U	5mL pH 9 No salt added	PP 3.5cm 0.6x0.2x0.2	Diphenyl ether	H_2SO_4 0.1M pH 1	Stirring 1300rpm 45°C 30min	FIA-DAD
[200]	Aristolochic acid	U	5mL pH 3	PP 3.3cm 0.6x0.2x0.2	1-octanol	NaOH 10mM pH 12	Stirring 800rpm 40min	LC-UV
[178]	Aconitine Hypaconitine Mesaconitine	U	5mL pH 11	PP 5.3cm 0.6x0.2x0.2	1-octanol	HCl 10mM pH 3	Stirring 800rpm 40°C 60min	LC-UV
[118]	Matrine Sophocarpine	U	4mL pH 13.7 No salt added	PP 7cm 0.6x0.2x0.2	1-octanol	H_3PO_4 100mM pH 1.5	Stirring 600rpm 60min	LC-UV
[35]	Clotrimazole Miconazole	P U	24mL pH 8 No salt added	PP 8cm 0.6x0.2x0.2	1-dodecane	Acetonitrile	Stirring 900rpm 40min	GC-FID
[119]	Propylthiouracil	P U	7.5mL pH 12 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol + 6% (w/v) Aliquat 336	$NaClO_4$ 2M pH 9	Stirring 1250rpm 25°C 40min	LC-UV
[120]	Dexamethasone	P U	7.5mL pH 3 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol + 5% (w/v) Aliquat 336	$NaClO_4$ 2M pH 9	Stirring 1250rpm Room temperature 60min	LC-UV
[121]	Desipramine	P U	8mL pH 13 No salt added	PP 5cm 0.6x0.2x0.2	Propyl benzoate	HCl 0.01M pH 2	Stirring 700rpm Room temperature 15min	Potentiometry
[122]	Mebendazole	P U	10mL pH 9 No salt added	PP 8.8cm 0.6x0.2x0.2	1-undecanol	HCl 100mM pH 1	Stirring 700rpm Room temperature 60min	LC-UV
[122]	Mebendazole	P U	7mL pH 1 No salt added	PP 8.8cm 0.6x0.2x0.2	NPOE	HCl 100mM pH 1	Stirring 700rpm Room temperature 150V 15min	LC-UV
[159]	Ephedrine	P U	7mL pH 11 NaCl 12% (w/v)	PP 7.5cm 0.6x0.2x0.2	Toluene + 10% (w/v) TEHP	HCl 1mM pH 3	Stirring 1200rpm Room temperature	LC-UV

							25min	
[159]	Ephedrine	P U	7mL pH 2	PP 7.5cm 0.6x0.2x0.2	NPOE + 10% (v/v) DEHP	HCl 100mM pH 1	Stirring 1000rpm Room temperature 100V 15min	LC-UV
[189]	Tramadol	P U	pH 11 Ionic strength 4M	PP 10cm 0.6x0.2x0.2	1-dodecane	Acetonitrile	Stirring 1000rpm 40min	GC-MS
[181]	Trimipramine Desipramine	P U	3mL pH *ca.* 12 5% (w/v) NaCl	PP 1.3cm 0.6x0.2x0.2	1-dodecane	Acetic acid 0.1M pH *ca.* 3	Stirring 860rpm 45ºC 20min	ESI-IMS
[160]	Naltrexone Nalmefene	P U	pH 2 pH *ca.* 10	PP 5.6cm 0.6x0.2x0.2	NPOE + DEHP (85:15 v/v)	HCl 100mM pH 1	Stirring 1250rpm 100V 20min	LC-UV
[123]	Pentazocine	P U	3mL pH 9 No salt added	PP 1.3cm 0.6x0.2x0.2	1-octanol	Acetic acid 0.5M pH *ca.* 3	Stirring 900rpm 20ºC 25min	ESI-IMS
[201]	Clomipramine	P U	3mL pH *ca.* 10 10% (w/v) NaCl	PP 3cm 0.6x0.2x0.2	1-dodecane	Methanol	Stirring 1700rpm 20min	CD-IMS
[124]	Alfentanil Fentanyl Sufentanil	P U	5mL pH *ca.* 10 No salt added	PP 3.5cm 0.6x0.2x0.2	Isoamyl benzoate	H_2SO_4 0.05M pH 1.3	Stirring 1200rpm 45ºC 20min	LC-UV
[125]	Amantadine	P U	3mL pH *ca.* 10 No salt added	PP 3cm 0.6x0.2x0.2	1-dodecane	Methanol	Stirring 1400rpm 20min	CD-IMS
[126]	Amphetamines	U	3mL pH 3 No salt added	PP 7cm 0.6x0.2x0.2	NPOE + 15% (v/v) TEHP	HCl 100mM pH 1	Stirring 1000rpm Room temperature 250V 7min	LC-UV
[127]	Thebaine	U	3mL pH 3 No salt added	PP 6cm 0.6x0.2x0.2	NPOE	HCl 100mM pH 1	Stirring 1250rpm 300V 15min	LC-UV
[99]	Atenolol Betaxolol Propranolol	OF	3mL pH 3 No salt added	PP 6cm 0.6x0.2x0.2	NPOE + 10% (v/v) DEHP + 5% (v/v) TEHP	HCl 100mM pH 1	Stirring 1250rpm 250V 15min	LC-UV
[100]	Levamisole	P U OF	4mL pH 2 No salt added	PP 9cm 0.6x0.2x0.2	NPOE + 5% (v/v) TEHP	HCl 100mM pH 1	Stirring 1000rpm 200V 15min	LC-UV
[169]	Atropine Scopolamine	P	pH 7.4	PP 10cm 0.55x0.45x0.18	1-heptanol:dimethyl benzene (30:70 v/v)	HCl 50mM pH 1.3	No forced convection 37ºC 5h	LC-UV
[128]	Nimesulide	P	5mL pH 2	PP 5.5cm	Dihexyl ether	NaOH 20mM pH 12.3	Stirring 400rpm	LC-UV

			No salt added				Room temperature (25°C) 30min	
[129]	Bisoprolol	P	5.6mL pH *ca.* 14 No salt added	PVDF 8.5cm 0.6x0.2x0.2	1-octanol	Formic acid 1M pH 1.8	Stirring 800rpm 35°C 25min	LC-FD
[185]	Imipramine Clomipramine	P U	2.1mL pH 4	PP 2.6cm 0.6x0.2x0.2	NPOE	pH 2	Stirring 1400rpm 200V 20min	GC-FID
[130]	Diclofenac	P U	2.1mL pH 11 No salt added	PP 3.1cm 1.2x0.2x0.2	1-octanol	NaOH 10mM pH 12	Stirring 1200rpm 30°C 20 V 5min	LC-UV
[90]	Butalbital Secobarbital Pentobarbital Phenobarbital	B	1mL pH *ca.* 1	PP 9cm 0.6x0.2x0.2	Decanol	NaOH pH 13	Sonication 5min	GC-MS
[34]	AMPAs MPA	U	3mL pH 1 30% (w/v) NaCl	PP 3cm 0.6x0.2x0.2	1-octanol	NaOH pH 14	Stirring 600rpm 42°C 50min	LC-MS
[38]	Methimazole	P U	7.5mL pH 12.2 CTAB 100mM No salt added	PP 8.8cm 0.6x0.2x0.2	Octanol	$NaClO_4$ 1.5M	Stirring 1250rpm 45°C 50min	LC-UV
[180]	Chloropheniramine Dextromethorphan	P	7.5mL pH 12.5 2% (w/v) NaCl	PP 8.8cm 0.6x0.2x0.2	Hexadecane	HCl 0.5mM pH 3.3	Stirring 1250rpm 60min	LC-UV
[175]	Basic drugs	P	50µL pH 7.4	PP 3cm 0.6x0.2x0.2 3 fibers	NPOE	Formic acid 10mM pH 2.9	No forced convection 200V 10min	LC-MS
[131]	Ofloxacin Ciprofloxacin	P	10mL pH 8.5 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol + 10% (w/v) Aliquat 336	pH 1 1mM NaCl	Stirring 1000rpm 45min	LC-UV
[132]	Trimipamine	P U	5mL pH 4.5 No salt added	PP 8cm 0.6x0.2x0.2	NPOE	pH 1	Stirring 1000rpm 51V 34min	CE-UV
[133]	Amitriptyline Imipramine Trimipramine Clomipramine	P U	5mL pH 12 No salt added	PP 8cm 0.6x0.2x0.2	1-dodecane	Methanol + 0.01M HCl pH 2	Stirring 1000rpm 40min	LC-UV
[134]	Mitiglinide	P U	10mL pH 1.5 No salt added	PP 6cm 0.6x0.2x0.2	1-octanol	NaOH 0.1M pH 13	Stirring 300rpm Room temperature 45min	LC-UV
[135]	Warfarin	P	11mL pH 2.3 No salt added	PP 8.8cm 0.6x0.2x0.2	1-octanol	0.1mM NaOH pH 11	Stirring 1000rpm 30min	LC-UV
[136]	Apigenin	U	11mL	PP	1-octanol	Carbonate 50mM	Stirring	LC-UV

			pH 3 No salt added	8.8cm 0.6x0.2x0.2		pH 11.5	1000rpm Room temperature 75min	
[137]	Amlodipine	P U	11mL pH 13 No salt added	PP 8.8cm 0.6x0.2x0.2	Dibenzyl ether	HCl 0.01M pH 2	Stirring 800rpm Room temperature 45min	LC-UV
[91]	Cathinone Amphetamines Ketamine DOI	B HB	80µL	PP 5cm 0.6x0.2x0.2	ENB	Acetic acid 10mM pH 3.4	No forced convection 15V 5min	LC-MS
[202]	Dextromethorphan Pseudoephedrine	P U	3mL pH 12.7 0% and 30% (w/v) NaCl for dextromethorphan and pseudoephedrine	PP 3cm 0.6x0.2x0.2	1-dodecane	Methanol	Stirring 750rpm 20min	CD-IMS
[138]	Hydroxyzine Cetirizine	P	10mL pH 5 → 11 No salt added	PP 8.2cm 0.6x0.2x0.2	1-octanol	pH 2	Stirring 1200rpm Room temperature 30min and then 20min	CE-UV
[101]	Amphetamines	H	50mg pH 14 1% (w/v) NaCl	PP 9cm 0.6x0.2x0.2	Dihexyl ether	HCl 0.1M pH 1	Vibrating 1000rpm 45min	GC-MS
[139]	Desipramine	P U	3mL pH *ca.* 13 No salt added	PP 0.8cm 0.6x0.2x0.2	1-dodecanol	Methanol	Stirring 900rpm Room temperature 25min	GC-NPD
[140]	Nalmefene Diclofenac	U	24mL Neutral pH (6.5) No salt added	PP 3.8cm 0.6x0.2x0.2 2 fibers	NPOE + 5% (v/v) DEHP 1-octanol	HCl 50mM pH 1.3 NaOH 50mM pH 12.7	Stirring 700rpm 40V Room temperature 14min	LC-UV
[170]	Pethidine Diphenhydramine Nortriptyline Methadone	U	1mL pH 12.6	PP 20mm 1.2x0.2x0.2	1-octanol	HCl 10mM pH 2	Vibrating 1000rpm 30min	DESI-MS
[141]	Sufentanil	P U	4mL pH 2.5 No salt added	PP 8cm 0.6x0.2x0.2	NPOE	HCl 0.1M pH 1	Stirring 1000rpm 190V 28min	Voltametry
[161]	Dexamethasone	P U	7.5mL pH 6	PP 3.3cm 0.6x0.2x0.2	1-octanol + 5% (w/v) Aliquat 336	$NaClO_4$ 0.65 M pH 10	Stirring 500rpm 80min	LC-UV
[142]	Metformin	P U	10mL pH 13.4 PFBC 10mg No salt added	PP 4cm 0.6x0.2x0.2	Dihexyl ether	HCl 100mM pH 1	Stirring 300rpm 70ºC 30min	LC-UV
[182]	NSAID	U	4mL pH 3 10% (w/v) NaCl	PP 4cm 0.6x0.2x0.2	Dihexyl ether	pH 13	Stirring 1500rpm 60ºC 45min	LC-UV

[143]	Venlafaxine and metabolites	P	4mL pH 10 No salt added	PP 15cm 0.6x0.2x0.2	1-octanol	Acetic acid 0.1M pH *ca.* 3	Stirring 1750rpm 20min	LC-MS/MS
[186]	Tolterodine	P U	3mL pH 2	PP 8cm 0.6x0.2x0.2	NPOE	HCl 500 mM pH 0.3	Stirring 1200rpm 54V 20ºC 24min	CE-UV
[203]	Ketoprofen	P	5mL pH 2 5% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	1-octanol	pH 11	Stirring 600rpm Room temperature 30min	LC-UV
[155]	Trimetazidine	P	2.1mL pH 14 250mM sodium 1-octanesulfonate 7% (w/v) Na_2SO_4	PP 10cm 0.6x0.2x0.2	1-octanol	HCl 0.5M pH 0.3	Stirring 600rpm 25min	LC-UV
[102]	Butalbital Secobarbital Pentobarbital Phenobarbital	L	1mL pH 1.1	PP 9cm 0.6x0.2x0.2	Eucalyptus oil	NaOH 0.1M pH 13	Sonicating 5min	GC-MS
[156]	NSAID	U	pH 2	PP 13cm 0.6x0.2x0.2	Dihexyl ether	pH 12	Stirring 300rpm 20min	CE-UV
[156]	Sulfonamides	U	50mL pH 4 28% (w/v) Na_2SO_4	PP 27cm 0.6x0.2x0.2	1-octanol	pH 12	Stirring 300rpm 6h	LC-UV LC-FD
[162]	Morphine	U	4mL pH 6	PP 8cm 0.6x0.2x0.2	NPOE + 10% (v/v) TEHP + 10% (v/v) DEHP	HCl 0.1M pH 1	Stirring 1000rpm 90V 24min	DPV
[173]	Ketamine Norketamine Dehydronorketamine	U	pH 10 10% (w/v) NaCl	PP 7.5cm 0.6x0.2x0.2	Eucalyptus essential oil	HCl 1 M pH 0	Vibrating 2400rpm 30min	GC-MS
[171]	Pyrethroid and metabolites	U	1.2mL pH 4 Conc. HCl 8.3% (w/v) NaCl	PP 1cm 1.67x0.52x0.33	Dihexyl ether	NaOH 0.1M pH 13	120min	LC-UV
[92]	TCA Desmethylclomipram in Fluoxetine Norfluoxetine	B	4mL pH *ca.* 13	PP 8cm 0.6x0.2x0.2	Dodecane	Formic acid 0.1M pH 2.4	Stirring 1200rpm 55ºC 30min	GC-MS
[144]	Dextromethorphan	P U	4mL pH 6 No salt added	PP 8cm 0.6x0.2x0.2	NPOE	HCl 0.1M pH 1	Stirring 1000rpm 110V 20min	DPV
[145]	Pramipexole	P U	10mL pH 11.5 No salt added	PP 4cm 0.6x0.2x0.2	1-octanol	pH 3	Stirring 600rpm 25ºC 40min	LC-UV
[146]	Methamphetamine Cocaine	U	4mL pH 6.5	PP 5cm	NPOE + 10% (v/v) DEHP + 10% (v/v) TEHP	pH 1 pH 12	Stirring 1000rpm	CE-UV

	Methadone Buprenorphine Morphine Ibuprofen Ketoprofen Enalapril		No salt added	0.6x0.2x0.2 4 fibers	1-octanol 1-octanol + 4% (w/v) CTAB NPOE		50V 15min	
[68]	Atorvastatin	U	24mL pH 4 1.2% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-octanol	NaOH 0.001M pH 11	Stirring 1000rpm 60min	LC-UV
[147]	Carbegoline	P U	15mL pH 10 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	pH 3	Stirring 750rpm Room temperature (25°C) 30min	LC-UV
[176]	Aristolochic acid I Aristolochic acid II	P	4mL pH 3 2% (w/v) NaCl	PP 6cm 0.6x0.2x0.2	1-octanol	pH 11	Stirring (magnetofluid) 2000rpm Room temperature (25°C) 8min	LC-FD
[148]	Berberine Palmatine	P U	3mL pH 11.8 No salt added	PP 8cm 0.6x0.2x0.2	1-octanol	HCl 0.1M pH 1	Stirring 600rpm 25°C 10min	CE-UV
[187]	Propranolol	P U	4mL pH 3.5	PP 8cm 0.6x0.2x0.2	NPOE	pH 1	Stirring 1250rpm 40V 20°C 32min	CE-UV
[191]	THC-COOH	U	1mL pH < 3 1% (w/v) NaCl	PP 9cm 0.6x0.2x0.2	Dihexyl ether	NaOH 0.1mM pH 10	Shaking 1200rpm 30min	GC-MS
[190]	Cocaine Ketamine Lidocaine	U	14mL pH 11 20% (w/v) NaCl	PP 10cm 0.6x0.2x0.2	1-dodecane	Acetonitrile	Stirring 700rpm 30min	GC-MS
[98]	Lidocaine	Se U	5mL pH *ca.* 12 1% (w/v) KCl	PP 8.2cm 0.6x0.2x0.2	1-octanol	HCl 0.01M pH *ca.* 2	Stirring 1000rpm Room temperature 50min	LC-UV
[149]	Rosiglitazone Metformin	P U	10mL → 10.7mL pH 9 → *ca.* 14 No salt added No derivitizing agent → 10mg/mL PFBC (100uL)	4cm	Dihexyl ether	HCl 0.1M pH 1	Stirring 300rpm Room temperature → 70°C 30 min+30min	LC-UV
[150]	Citalopram	U	4mL pH 12.5 No salt added	PP 8.0cm 0.6x0.2x0.2	1-octanol	pH 2.2	Stirring 1000rpm 28min	CE-UV
[70]	Triamterene	U	24mL pH 14 11% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-decanol	pH 1	Stirring 800rpm 90min	LC-UV
[151]	Olanzapine Fluoxetine	U P	3mL pH 12 5% (w/v) NaCl	PP 2.8cm 1.2x0.2x0.2	1-octanol	HCl 25 or 10mM pH 1.6 or 2	Stirring 1000rpm 60min	LC-UV
[151]	Olanzapine Fluoxetine	U P	3mL pH 6	PP 2.8cm	NPPE	HCl 10mM pH 2	Stirring 1000rpm	LC-UV

			No salt added	1.2x0.2x0.2			200V 30min	
[179]	Benzodiazepines	U	2mL pH 10 10% (w/v) NaCl	PP 9cm 0.6x0.2x0.2	Dihexyl ether:1-nonanol (9:1 v/v)	HCl 3M pH 0	Vibrating 2400rpm 90min	GC-MS
[152]	Diclofenac Naproxen	U P	4mL pH 7.4 Triton X-100 0.2mM No salt added	PP 6cm 0.6x0.2x0.2	1-octanol	pH 12	Stirring 1000rpm 15V 15min	CE-UV
[188]	Phenazopyridine	U P	6.5mL Neutral pH	PP 7.5cm 0.6x0.2x0.2	NPOE	HCl 100mM pH 1	Stirring 1250rpm 100V 20min	LC-UV
[172]	Atrazine and degradation products	U	200mL pH 7 20% (w/v) NaCl	PP 20cm 0.28x0.05x0.1	Dihexyl ether	HCl 1M pH0	Stirring 150rpm Room temperature (*ca.* 20) 5h	LC-UV
[183]	Sitagliptin	U	15mL pH 10.5 35% (w/v) NaCl	PP 8.5cm 0.6x0.2x0.2	1-octanol	pH 3	Stirring 1000rpm 25°C 50min	LC-UV
[153]	Atorvastatin Lovastatin Simvastatin	U	18mL pH 2 No salt added	PP 10cm 0.6x0.2x0.2	1-dodecane + 5% (w/v) TOPO	Methanol + NaOH 0.1M pH 13	Stirring 1000rpm 45min	LC-UV GC-FID
[163]	Levonogestrel Megestrol	U	20mL No pH adjustment 10% (w/v) NaCl	PP 8cm 0.6x0.2x0.2	1-dodecane + 5% (w/v) TOPO	Methanol	Stirring 1000rpm 40min	LC-UV
[204]	SSRI	P U	6mL pH 12.8 2% (w/v) NaCl	PP 3cm 0.6x0.2x0.2	Phenetole	Acetic acid 0.1M pH *ca.* 3	Stirring 1000rpm 40min	Sweeping-MEKC
[77]	Albendazole Triclabendazole	U	6mL pH 8 No salt added	PP 8.8cm 0.6x0.2x0.2	1-undecanol	1-undecanol	Vibrating 3min	LC-FD
[95]	Codeine Naproxen Ketamine Ibuprofen	P M	4mL pH 6	PP 2.1cm 1.2x0.3x0.2	1-octanol (acidic) 2-ethyl hexanol (basic)		Stirring 750rpm 175V 25min	LC-UV
[95]	Codeine Naproxen Ketamine Ibuprofen	P M	4mL pH 6	PP 2.1cm 1.2x0.3x0.2	1-octanol (acidic) 2-ethyl hexanol (basic)	HCl 32mM pH 1.5 (basic) NaOH 32mM pH 12.5 (acidic)	Stirring 750rpm 150V 6min 400V 19min	LC-UV
[154]	Clozapine	P	30mL pH 4.5 No salt added	PP 10cm 1.2x0.3x0.2	NPOE	pH 4.5	Stirring 1000rpm 200V 18min	Voltametry
[164]	Nalidixic acid	U	5mL pH *ca.* 12	PP 10cm 0.6x0.2x0.2	1-octanol + Aliquat 336 (9:1 v/v)	NaCl 1M	Stirring 1000rpm *ca.* 25°C 45min	LC-UV
[96]	Ketamine (B) Codenie (B)	P M	4mL pH 6	PP 2.1cm	2-ethyl hexane (B) 1-octanol (A)	HCl pH 1.5 (B) NaOH pH 12.5 (A)	Stirring 750rpm	LC-UV

	Naproxene (A) Ibuprofen (A)			1.2x0.3x0.2			150V 6min (A) → 400V 19min (B)	
[104]	Lamotrigine	P	4mL pH 9 No salt added	PP 15cm 0.6x0.2x0.2	1-octanol	HCl pH 4	Stirring 500rpm 30min Room T	CE-UV
[79]	Oxazepam Lorazepam	U P	25mL No pH adjustment	PP 7cm 0.6x0.2x0.2	n-dodecane + TOPO 7.5% (w/v)	Acetonitrile	Stirring 1000rpm 30min	LC-MS
[103]	Muscimol Tryptophan Tryptamine	U	10mL pH 4 No salt added	PP 8cm 0.6x0.2x0.2	Dihexyl ether + DEHPA 20% (w/w)	HCl 200mM	Stirring 800rpm 60min	LC-UV
[80]	Triamterene	U	24mL NaOH 3M 2M NaCl	PP 8.5cm 0.6x0.2x0.2	1-decanol	HCl pH 1	Stirring 800rpm 90min	LC-UV
[105]	Ibuprofen Diclofenac	U	4mL pH 10.5 No salt added	PP 6cm 0.6x0.2x0.2	1-octanol + 0.6% w/v C60 fullerene	NaOH pH 12.8	Stirring 1000rpm 28min 6V	LC-UV
[165]	Bismuth	P	5mL 5mM H_2SO_4	PP 3.5cm 0.6x0.2x0.2	1-octanol + 1% (v/v) DEHP	300mM H_2SO_4	Stirring 700rpm 10min 70V	UV-Vis
[106]	Diclofenac	U P	0.05M HCl No salt added	PP 2.5cm 0.6x0.2x0.2	n-dodecane	Methanol	Stirring 1000rpm 20min Room T	ESI-IMS

Table 3. 3-phase HF-LPME (and variants) of drugs of forensic interest in biological matrices. The concentration values of salt added were converted to % (w/v); the pH were calculated based on the concentration of base or acid in some cases. Abbreviations: (A) = acidic; Aliquat-336 = 3-caprylil methyl ammonium chloride; AMPAs = alkyl methylphosphonic acids; (B) = basic; B = whole blood; *ca*. = approximately; CE = capillary electrophoresis; DEHP = di-(2-ethylhexyl) phosphate; DOI = 2,5-dimethoxy-4-iodoamphetamine; FD = fluorescent detector; FDNB = 1-fluoro-2,4-dinitrobenzene; FIA = flow injection analysis; FID = flame ionization detector; GC = gas chromatography; H = hair; HB = haemolysed blood; id = internal diameter; L = liver; LC = high performance liquid chromatography; M = breast milk; MEKC = micellar electrokinetic chromatography; MPA = methylphosphonic acid; MS = mass spectrometry; MS/MS = tandem mass spectrometry; n.r. = not reported; Na_2SO_4 = sodium sulfate; NaCl = sodium chloride; NDCIT = N-desmethylcitalopram; NPD = nitrogen-phosphorus detector; NPOE = 2-nitrophenyl octylether; OF = oral fluid; P = plasma; PP = polypropylene; ps = pore size; PVDF = polyvinylidene difluoride; Se = serum; SSRI = selective serotonin reuptake inhibitors; T = temperature; THC-COOH = 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid; TCA = tricyclic antidepressants; TEHP = tris(2-ethylhexy)phosphate; TOPO = trioctylphosphine oxide; wt = wall thickness; U = urine; UV = ultra-violet; Vis = visible

Please the following Figures should be printed in color:

- Figure 1
- Figure 3

Thank you,

Rafael